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Weekly records of feed consumption and weight of the birds were kept for the 25-bird series of pens. Records were kept for 4-week periods for the 15-bird series. All feed consumption was figured on the basis of hen-days, and an allowance was made for the feed eaten by the cockerels. Each egg was weighed on the day that it was laid. The experiment covered a period of 344 days; it was begun on September 3, 1929, and terminated on August 12, 1930.

### EXPERIMENTAL RESULTS

#### EFFECT OF DIET ON AGE AT WHICH FIRST EGG WAS LAID

The pullets used were approximately of the same age. Distribution in the several pens according to weight, so that no significant differences existed between the mean weights of the birds in any two pens (Table 3), probably resulted in there being no significant differences in age between the birds in any two pens.

The difference between the average time before the first egg was produced by birds receiving the basal diet and those receiving each of the other diets is significant only in the case of diet 7, which contained cottonseed meal as a The weights of the birds on diet 7 fell below the her diets at about the time the average bird on to lay. The feed consumption of the birds on eat of the birds on any of the other diets at this ee (15) found that cottonseed meal apparently his lack of pala- tability may account for the significantly greater age at first egg of the birds on diet 7 in the present experiment.

TABLE 3.—Average number of days before first egg, and average initial, maximum, and final live weights of the birds on each diet

Diet No.	Average number of days before first egg	Difference in time before first egg of birds on indicated diet and on diet 9	Average initial weight of birds	Difference in average initial weight of birds on indicated diet and on diet 9	Average maximum weight of birds	Difference in average maximum weight of birds on indicated diet and on diet 9	Average final live weight of birds	Difference in average final weight of birds on indicated diet and on diet 9
	Days	Days	Grams	Grams	Grams	Grams	Grams	Grams
1.....	79.4±3.91	-14.1±6.0	1,770±30.2	+48±39.0	2,691±38.3	-2±54.9	2,437±41.1	+316±53.0
2.....	90.3±6.01	-3.2±7.0	1,748±24.8	+26±35.1	2,689±30.7	-4±49.9	2,373±37.6	+252±50.3
3.....	96.5±4.90	+3.0±6.7	1,785±25.3	+63±35.4	2,799±31.1	+106±50.2	2,462±36.8	+341±49.7
4.....	80.3±4.51	-13.2±6.0	1,771±21.0	+49±32.5	2,757±36.8	+64±53.9	2,479±40.2	+358±52.3
5.....	80.5±4.53	-13.0±6.1	1,807±22.7	+85±33.6	2,650±38.3	-43±54.9	2,106±33.4	-15±47.2
6.....	83.5±4.31	-10.0±6.6	1,808±26.3	+80±36.1	2,681±28.5	-12±48.6	2,417±38.3	+256±50.8
7.....	119.3±6.30	+25.8±7.3	1,761±25.9	+39±35.9	2,618±35.4	-75±52.9	2,202±33.1	+81±47.0
8.....	89.4±5.20	-4.1±7.08	1,728±23.3	+6±34.0	2,794±30.2	+101±49.6	2,486±30.1	+365±45.0
9.....	93.5±4.81	-----	1,722±24.8	-----	2,693±39.4	-----	2,121±33.4	-----

There is no evidence of correlation between the number of days before first egg and the percentage of protein in the diet. Even the basal diet contained sufficient protein to permit normal assumption of egg laying.

#### EFFECT OF DIET ON WEIGHT OF BIRDS

The average maximum weights, shown in Table 3, were observed on February 19, except those of the birds on diet 1, which were at-

tained about a month later. The differences in average approximate maximum weight between the birds on diet 9 and those on the other diets are not significant. The average maximum weights of the birds on diet 5 and on diet 7 were significantly lighter than those of the birds on diet 3. The difference between the average maximum weight of the birds on diet 5 and those on diet 3 was  $149 \pm 41.0$  g and between the average for those on diet 7 and those on diet 3,  $181 \pm 47.1$  g. Both of these differences are more than three times their probable errors and therefore are significant.

The birds on diets 1, 2, 3, 4, 6, and 8 had significantly heavier final live weights than those on diet 9. The weights began to diverge when laying commenced and the differences in weight became greater as the experiment progressed. A comparison of the final live weights (Table 3) with percentage of protein in the diets (Table 2) shows a pronounced positive correlation between final live weight and percentage of protein in the diet. The value of this correlation is  $r = +0.355 \pm 0.0341$ , and being ten times its probable error, it is very significant. This means that pullets laying eggs are able to maintain their weight more readily on diets containing a high percentage of protein, at least within the limits of the percentages used in this experiment. On diet 4 the weight of the birds was more effectively maintained than would have been expected from its protein content.

#### EFFECT OF DIET ON WEIGHT OF EGGS

The average egg weights for the hens on diets 1, 3, 4, 6, and 8 are significantly greater than the average egg weight for the hens on diet 9. (Table 4.) A comparison of the values for egg weight with the percentage of protein in each diet shows that they are positively correlated. The coefficient of correlation is  $r = +0.339 \pm 0.0317$ , which, being more than ten times its probable error, is very significant. Here, too, diet 4 was more efficient than would be expected from its protein content. The eggs from the hens on this diet were heavier than those from the hens on diets 2 and 7, which had the same percentage of protein. They were as heavy as those from the hens on diet 6, which had 3 per cent more protein.

TABLE 4.—Average weight and total number of eggs produced by the hens on each diet \*

Diet No.	Average egg weight	Difference in average weight of eggs from birds on indicated diet and on diet 9	Total eggs produced	Average hen-days	Birds alive August, 1930
	Grams	Grams	Number	Number	Number
1.....	57.8 $\pm$ 0.378	+2.9 $\pm$ 0.550	5,154	327.1 $\pm$ 4.98	54
2.....	56.1 $\pm$ .394	+1.2 $\pm$ .561	4,917	328.4 $\pm$ 3.53	55
3.....	59.1 $\pm$ .388	+4.2 $\pm$ .557	4,818	320.3 $\pm$ 6.44	53
4.....	57.3 $\pm$ .371	+2.4 $\pm$ .545	4,493	323.6 $\pm$ 4.69	30
5.....	54.9 $\pm$ .350	$\pm 0 \pm$ .531	3,764	324.6 $\pm$ 5.28	33
6.....	57.0 $\pm$ .367	+2.1 $\pm$ .542	4,837	331.6 $\pm$ 5.45	34
7.....	56.0 $\pm$ .359	+1.1 $\pm$ .537	3,540	325.4 $\pm$ 7.57	37
8.....	58.3 $\pm$ .347	+3.4 $\pm$ .529	5,719	337.4 $\pm$ 3.22	38
9.....	54.9 $\pm$ .399		4,177	335.9 $\pm$ 2.07	33

\* All averages given are unweighted averages of the average for each hen in each pen.

Egg weight is also positively correlated with body weight. In the present experiment the correlation coefficient for egg weight and body weight is  $r = +0.454 \pm 0.0309$ .

Since the diet affects the weight of the egg, it affects the weight of the yolk or albumen, or both. The values of yolk weight and albumen weight given in Table 5 are unweighted averages of the average of one to five eggs for each hen, taken at different times throughout the year, and are therefore only approximately representative. The average yolk weights for the birds on diets 1, 3, and 8 are significantly greater than the average yolk weight for the birds on diet 9. The weights of albumen in the eggs from the birds on diets 1, 3, 4, 6, 7, and 8 are significantly greater than those in the eggs from the birds on diet 9.

TABLE 5.—Average weight of yolk and albumen of eggs from hens on each diet

Diet No.	Average yolk weight	Difference in yolk weight of eggs from birds on indicated diet and on diet 9	Average albumen weight	Difference in albumen weight of eggs from birds on indicated diet and on diet 9
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
1.....	17.09±0.212	+1.19±0.274	32.24±0.401	+2.33±0.563
2.....	16.06±.186	+ .16±.254	31.07±.359	+1.16±.534
3.....	16.81±.198	+ .91±.263	32.11±.401	+2.20±.563
4.....	16.11±.188	+ .21±.253	31.71±.352	+1.80±.529
5.....	15.95±.291	+ .05±.339	30.25±.339	+ .34±.520
6.....	16.59±.166	+ .69±.240	31.72±.305	+1.81±.498
7.....	16.06±.225	+ .16±.284	31.75±.458	+1.84±.605
8.....	16.68±.160	+ .78±.235	32.21±.318	+2.30±.507
9.....	15.90±.173	-----	29.91±.395	-----

The coefficient of correlation between yolk weight and egg weight is  $r = +0.405 \pm 0.0326$ , and between albumen weight and egg weight,  $r = +0.759 \pm 0.0165$ . There is, of course, a significant correlation between yolk weight and albumen weight, but the coefficient is surprisingly low:  $r = +0.312 \pm 0.0220$ . The coefficient of correlation between percentage of protein in the diet and albumen weight is  $r = +0.148 \pm 0.0384$ , and between percentage of protein and yolk weight is  $r = +0.212 \pm 0.0374$ . These values are probably low because of the small number of each hen's eggs used as a sample.

#### EFFECT OF DIET ON INTENSITY OF PRODUCTION

The total number of eggs produced by the hens on each diet is given in Table 4. These figures do not present a true picture of the production of the individual hens, since they are not corrected for mortality and other factors. The number of deaths varied from 2 on diet 8 to 10 on diet 4 (Table 4), but the difference in mean days per hen for these two diets,  $13.8 \pm 5.69$ , is not significant. The hens were apparently able to survive about equally well on each of the diets. The values for intensity of production, obtained in the manner indicated and shown in Table 4, are too low. The pullets were on the diets for some time before they commenced to lay. The efficiency of the diets for sustaining production is more adequately shown in Table 6. The true values for yearly egg production, however, would

be somewhat lower than those in Table 6, because percentage of egg production usually drops toward the end of the first laying year, which the experiment did not include. The birds on diets 1, 2, 3, and 8, as shown in this table, laid at a significantly greater rate than those on diet 9.

TABLE 6.—Average annual egg production of hens on each diet <sup>a</sup>

Diet No.	Production based on average number of hen-days after first egg	Difference in average production of birds on indicated diet and on diet 9	Eggs per hen per pullet year (calculated on production after first egg)	Diet No.	Production based on average number of hen-days after first egg	Difference in average production of birds on indicated diet and on diet 9	Eggs per hen per pullet year (calculated on production after first egg)
	<i>Per cent</i>	<i>Per cent</i>	<i>Number</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Number</i>
1.....	53.1±2.15	+10.0±2.65	197.0	6.....	47.3±1.88	+4.2±2.44	173.0
2.....	51.3±2.08	+8.2±2.60	187.5	7.....	43.6±1.71	+1.5±2.31	159.5
3.....	51.6±2.03	+8.5±2.55	188.1	8.....	55.9±1.72	+12.8±2.32	204.5
4.....	46.0±2.30	+2.9±2.77	168.0	9.....	43.1±1.55	-----	157.8
5.....	39.9±1.22	-3.2±1.97	145.8				

<sup>a</sup> The averages given in this table are unweighted averages of the average for each hen in each pen.

The data collected in the present experiment show positive correlation,  $r = +0.226 \pm 0.0347$ , between percentage of protein in the diet and percentage of production after first egg, in spite of the fact that proteins of different origin were used. The correlation is considerably lower than that reported by Kempster (14),  $r = +0.5179 \pm 0.071$ , but he correlated pen averages, whereas the present correlation is for individual hens. In his experiments different percentages of the same protein concentrate, meat meal, were used.

## EFFECT OF DIET ON WINTER EGG PRODUCTION

From the economic standpoint, it is necessary that a diet support winter egg production. The number of eggs produced before March 3 and the percentage which they are of the total eggs produced are given in Table 7.

TABLE 7.—Winter egg production of hens on each diet

Diet No.	Eggs laid from September 4 to March 3		Diet No.	Eggs laid from September 4 to March 3	
	Number	Per cent of total		Number	Per cent of total
1.....	2,046	39.5	6.....	1,759	36.5
2.....	1,466	29.8	7.....	885	25.0
3.....	1,684	35.0	8.....	1,919	33.4
4.....	1,948	43.4	9.....	1,571	37.5
5.....	1,464	39.0			

The birds on diet 7 produced few winter eggs in proportion to the total number produced. The birds on diet 4 laid more eggs during the winter than would be expected from the protein content of the diet, but in the spring and summer this diet proved distinctly less efficient, which confirms the results of Pederick and Clark (25).

EGG PRODUCTION PER UNIT WEIGHT OF FEED AND OF PROTEIN

Table 8 gives the egg production per gram of feed and per gram of protein eaten. The supplements used increase in efficiency rather regularly with increase of protein content. The supplement in diet 8 is more efficient than would be expected from the protein content of the three concentrates of which it was composed. This increased efficiency is attributed to the mutual supplementary action of these concentrates.

TABLE 8.—Egg production of hens, per unit weight of feed and of protein, when on each diet

Diet No.	Eggs produced per hen per day after average date of first egg	Feed per hen per day after first egg	Eggs produced per gram of feed eaten	Eggs <sup>a</sup> produced per gram of supplement eaten	Eggs produced per gram of protein eaten	Eggs <sup>a</sup> produced per gram of supplemental protein eaten
	Grams	Grams	Gram	Gram	Grams	Gram
1.....	30.7	134.2	0.229	0.380	0.990	0.585
2.....	28.9	136.8	.212	.263	1.348	.828
3.....	31.1	134.9	.231	.357	1.012	.598
4.....	26.4	128.6	.205	.235	1.282	.775
5.....	21.8	126.6	.172	.073	1.540	.731
6.....	26.9	122.8	.219	.305	1.198	.671
7.....	24.4	127.8	.191	.167	1.190	.700
8.....	32.6	128.8	.251	.474	1.222	.831
9.....	23.6	119.2	.198	.....	1.720	.....

<sup>a</sup> Calculated by use of the efficiency basal ration.

<sup>b</sup> Efficiency of supplement in diet 8 calculated from the efficiencies of supplements in diets 1, 3, and 4 is 0.333; the difference, 0.141, is attributed to the supplementary action of the three concentrates on one another.

The efficiency of the protein decreases as the percentage fed increases. The efficiency of the protein in the supplement of diet 8 is somewhat higher than would be expected from the percentage fed. The efficiency of the protein of the supplement in diet 2 is worthy of note. It gave 0.828 g of egg per gram of supplemental protein eaten as compared with 0.775 g per gram of supplemental protein for the supplement in diet 4.

Egg production per gram of feed eaten plotted against percentage of protein in the diet is shown in Figure 1. Figure 2

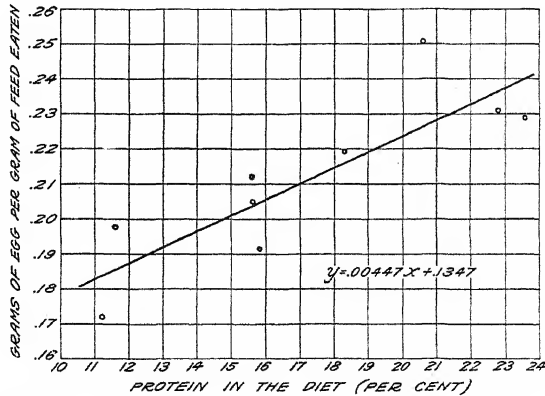


FIGURE 1.—Increase in egg production per gram of feed eaten with increased percentage of protein in the diet. The curve represents the values given by the least square solution of the formula  $Y = ax + b$ , in which  $Y$  = grams of egg per gram of feed eaten,  $x$  = percentage of protein in the diet, and  $a$  and  $b$  = constants. The  $o$ 's represent the observed values

shows the egg production per gram of protein eaten plotted against percentage of protein in the diet. It has been pointed out that Norris and Heuser (22), in feeding experiments with growing chicks, found a

decreased efficiency of protein with increased protein in the diet. Their hypothesis, that the protein of meat meal, which they used as a supplement, was deficient in essential amino acids, certainly does not explain the decreasing efficiency of protein with increased protein level in egg production.

The data plotted in Figure 2 are taken from 8 experiments carried out in 6 States with 3 breeds of chickens. Twelve protein supplements were used: Meat meal of two kinds, tankage, fish meal, dried buttermilk, sour skim milk, whey, crab scrap, yeast product, cottonseed meal, soybean meal, pea meal, combinations of several of those listed, and several unsupplemented cereal diets. The low values for the basal rations of Lee,<sup>3</sup> Kempster (14), and Philips (27) are more probably due to the low feed consumption of the birds on these

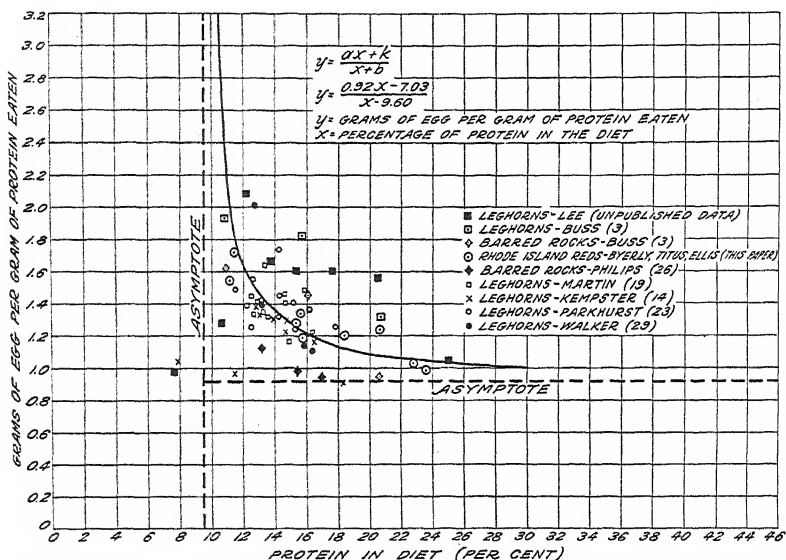


FIGURE 2.—Decrease of protein efficiency with increased protein content in the diet. The formula given describes an equilateral hyperbola and was fitted only to the data in the present experiment by the method of least squares. For further explanation see text.

diets than to a relatively low protein efficiency, for in the present experiment the basal ration had the highest protein efficiency of any of the diets used. Feed consumption of birds on this diet was practically the same as that on the other diets, whereas the feed consumption of Lee's birds was very low. Some of the higher values obtained when between 15 and 20 per cent of protein was fed are probably due to stimulated feed consumption rather than to high protein efficiency. In general, the diets containing milk have a somewhat higher protein efficiency for the percentage used than those that do not contain milk. It is remarkable that these data from such widely scattered sources should show so great a degree of similarity.

The curve plotted is fitted only to the data for the present experiment and no rational significance is implied. It seems certain that for diets on which the feed consumption is approximately equal per

<sup>3</sup> LEE, A. R. Unpublished data. 1925.

unit weight of bird, the efficiency of any protein for egg production decreases with increased protein content in the diet, at least between the limits of 11 and 24 per cent. Mitchell (21) found that the biological value of proteins decreases with increased percentage of protein in the diet, regardless of protein intake, in the case of milk, corn, and oat proteins fed to rats. Since this is apparently true for proteins generally fed to laying hens, Norris and Heuser's (22) hypothesis can not hold. Mitchell disposes of the argument which they advance in the paper cited. The following hypothesis for the decreased efficiency is very similar to Mitchell's. Specific dynamic action may account for part of the decrease. It may be that there is a threshold value for amino acids in relation to concentration of other nutrients in the blood and body fluids above which they are deaminized. If this be true, it would follow that above the threshold value more and more of the amino acids would be destroyed, and relatively less, though in absolute terms more, would pass into the egg. The efficiency need not decrease as the maintenance value is approached, for a hen may lay eggs for a time on a submaintenance ration, as is shown by Titus (28).

#### CORRELATION BETWEEN EGG-PRODUCTION FACTORS

It has been shown that within the limits of the protein levels used in this experiment, percentage of protein is positively correlated with factors in egg production such as intensity of laying, egg weight, yolk weight, and possibly albumen weight. Significant positive correlation exists between percentage of protein in the diet and body weight; body weight is closely correlated with egg weight.

Table 9 shows that egg weight and percentage of protein in the diet, egg weight and final body weight, egg weight and yolk weight, and egg weight and albumen weight are positively and significantly correlated, as has been pointed out. The correlation between egg weight and intensity of production is positive but not significant in contrast to Jull's (12) report of negative correlation between these factors. Final body weight, intensity, yolk weight, and possibly albumen weight, are positively and significantly correlated with percentage of protein in the diet. Yolk weight, albumen weight, and intensity are positively correlated with final body weight. Yolk weight and albumen weight are also positively and significantly correlated.

TABLE 9.—Zero-order correlations between the factors in egg production affected by protein content of the diet

Factor No.	Egg-production factor	Correlation between factors indicated				
		2	3	4	5	6
1	Egg weight.....	+0.339±0.0317	+0.454±0.0309	+0.087±0.0366	+0.405±0.0326	+0.759±0.0165
2	Percentage of protein.....		+0.355±0.0341	+0.226±0.0347	+0.212±0.0374	+0.148±0.0384
3	Final body weight.....			+0.149±0.0360	+0.274±0.0360	+0.298±0.0360
4	Intensity <sup>a</sup> .....				+0.053±0.0391	+0.002±0.0385
5	Yolk weight.....					+0.312±0.0220
6	Albumen weight.....					

<sup>a</sup> "Intensity" is used as a brief term denoting percentage of production after the average time the hens on a diet had laid their first eggs.

Zero-order correlations may be only apparent. For example, the correlation between egg weight and protein may be present only because of the effect of percentage of protein on body weight, since egg weight and body weight are closely correlated. To get a more nearly accurate picture, it is necessary to find the net correlations; these are given in Table 10.

TABLE 10.—*Net correlations of factors in egg production*

Factor No.	Factors in egg production	Net correlation coefficients
<i>r</i> <sub>12,24</sub> .....	Egg weight and percentage of protein, with body weight and intensity held constant.	+0.219±0.0357
<i>r</i> <sub>13,24</sub> .....	Egg weight and body weight, with percentage of protein and intensity held constant.	+ .380± .0321
<i>r</i> <sub>14,23</sub> .....	Egg weight and intensity, with percentage of protein and body weight held constant.	-.019± .0375
<i>r</i> <sub>15,234</sub> .....	Egg weight and yolk weight, with percentage of protein, body weight, and intensity held constant.	+ .361± .0327
<i>r</i> <sub>16,2345</sub> .....	Egg weight and albumen weight, with percentage of protein, body weight, intensity, and yolk weight held constant.	+ .736± .0171
<i>r</i> <sub>23,14</sub> .....	Percentage of protein and body weight, with egg weight and intensity held constant.	+ .222± .0357
<i>r</i> <sub>24,13</sub> .....	Percentage of protein and intensity, with egg weight and body weight held constant.	+ .187± .0363
<i>r</i> <sub>25,24</sub> .....	Percentage of protein and yolk weight, with body weight and intensity held constant.	+ .128± .0369
<i>r</i> <sub>26,245</sub> .....	Percentage of protein and albumen weight, with body weight, intensity, and yolk weight held constant.	-.006+ .0375
<i>r</i> <sub>34,12</sub> .....	Live weight and intensity, with egg weight and percentage of protein held constant.	+ .078± .0372
<i>r</i> <sub>35,24</sub> .....	Body weight and yolk weight, with percentage of protein and intensity held constant.	+ .218± .0357
<i>r</i> <sub>36,245</sub> .....	Body weight and albumen weight, with percentage of protein, intensity, and yolk weight held constant.	+ .232± .0354
<i>r</i> <sub>45,23</sub> .....	Intensity and yolk weight, with percentage of protein and body weight held constant.	-.011± .0375
<i>r</i> <sub>46,235</sub> .....	Intensity and albumen weight, with percentage of protein, body weight, and intensity held constant.	-.054± .0375
<i>r</i> <sub>56,234</sub> .....	Yolk weight and albumen weight, with percentage of protein, body weight, and intensity held constant.	+ .244± .0354

The net correlations show that egg weight, final body weight, and intensity are positively and significantly correlated with percentage of protein in the diet. There is a slight correlation between yolk weight and percentage of protein but none between albumen weight and percentage of protein. Intensity of production is correlated significantly only with percentage of protein in the diet. The correlation coefficients between intensity and egg weight, yolk weight, and albumen weight are all negative, but none of them is significant. This stock has been selected for several generations for both egg weight and intensity.

The primary purpose here is to find how much one may expect to increase the production of eggs, thereby decreasing the feed cost of egg production, by increasing the protein content of the diet within the limits of the percentages used in the experiment. Percentage of protein is an independent variable and egg weight, body weight, and intensity are the dependent variables with respect to percentage of protein. The net correlations for these factors may be interpreted in terms of changed egg production or feed cost of egg production by using the regression equation of the dependent on the independent variables in each case and summing the results. The regression of egg weight on body weight, intensity of production on body weight, and egg weight on intensity of production must also be used.



The type equation is

$$Y - \bar{Y} = r \frac{\sigma_Y 1.234}{\sigma_X 2.134} (X - \bar{X}),$$

in which  $Y$  is the value of the dependent variable corresponding to a selected value of the independent variable  $X$ ,  $\bar{Y}$  is the mean value for the dependent variable,  $r$  is the net correlation coefficient,  $\sigma_Y$  is the standard deviation about  $\bar{Y}$ ,  $\sigma_X$  is the standard deviation about  $\bar{X}$ , when egg weight, intensity, and body weight are constant.  $\bar{X}$  is the mean for the values of the independent variable and  $X$  the selected value for the independent variable.

If the lowest and highest observed or derived values for the independent variables are used, the demonstration may be made by the following use of the regression equations.

For egg weight ( $Y$ ) on percentage of protein ( $X$ ):

$$Y = 0.187X + 53.66.$$

When  $X = 11.2$  per cent,  $Y = 55.75$  g, and when  $X = 23.6$  per cent,  $Y = 58.07$  g, the increment being +2.32 g----- (a)

For body weight ( $Y$ ) on percentage of protein ( $X$ ):

$$Y = 17.8X + 20.3.$$

When  $X = 11.2$  per cent,  $Y = 2,219$  g, and when  $X = 23.6$  per cent,  $Y = 2,440$  g, the increment being +221 g----- (b)

For egg weight ( $Y$ ) on the derived body weight ( $X$ ):

$$Y = 0.00407X + 46.38.$$

When  $X = 2,229$  g,  $Y = 55.45$  g, and when  $X = 2,450$  g,  $Y = 56.35$  g, the increment being +0.90 g----- (c)

For intensity of production ( $Y$ ) on percentage of protein ( $X$ ):

$$Y = 0.825X + 33.91.$$

When  $X = 11.2$  per cent,  $Y = 43.15$  per cent, and when  $X = 23.6$  per cent,  $Y = 53.38$  per cent, the increment being +10.23 per cent----- (d)

The coefficients of correlation between intensity and egg weight and between body weight and intensity are very small, and since they are not significant might be ignored, but for the sake of completeness they are given.

For intensity of production ( $Y$ ) on body weight ( $X$ ):

$$Y = 0.00431X + 38.09.$$

When  $X = 2,229$  g,  $Y = 47.70$  per cent, and when  $X = 2,450$  g,  $Y = 48.65$  per cent, the increment being +0.95 per cent---- (e)

For egg weight ( $Y$ ) on intensity of production ( $X$ ):

$$Y = -0.0037X + 57.05.$$

When  $X = 42.25$  per cent,  $Y = 56.89$  g, and when  $X = 53.38$  per cent,  $Y = 56.85$  g, the increment being -0.04 g----- (f)

Thus, increasing the protein content from 11.2 to 23.6 per cent would be expected to increase the annual mean egg weight by 2.32 g (a) by direct effect and by 0.90 g (c) indirectly by increasing body weight. The increase in protein would increase the intensity of production by 10.23 per cent (d), and the increase in body weight would increase the intensity by 0.95 per cent (e), making a net increase of 11.18 per cent as compared with the observed increase of 13.2 per cent between diet 5 and diet 1. (Table 6.) The increase in intensity would tend to decrease weight by 0.04 g (f), leaving a net increase in egg weight of 3.18 g as compared with the observed increase of 2.9 g. (Table 4.) Intermediate values may be interpolated. It probably would not be fair to extrapolate beyond the range of the protein percentages used

in the present experiment, because it is probable that the efficiency of feed containing increased increments of protein in the diet increases according to a hyperbolic curve rather than a straight line. Linear regression gives a fair fit only because the range of values is fairly narrow.

The other net correlations may be evaluated in the same manner. It should be noted that although the net correlation between percentage of protein in the diet and each of the several factors in egg production is in no case high, the total effect of increasing protein is very important.

The multiple correlations existing between the several factors studied are given in Table 11.

TABLE 11.—Multiple correlation coefficients of the factors in egg production

Factor No.	Factors in egg production	Multiple correlations
<i>r</i> <sub>1,234</sub> .....	Egg weight with percentage of protein, body weight, and intensity.....	+0.492±0.0285
<i>r</i> <sub>2,134</sub> .....	Percentage of protein with egg weight, body weight, and intensity.....	+0.442±0.0303
<i>r</i> <sub>3,124</sub> .....	Live weight with egg weight, percentage of protein, and intensity.....	+0.519±0.0297
<i>r</i> <sub>4,123</sub> .....	Intensity with egg weight, percentage of protein, and body weight.....	+0.239±0.0354
<i>r</i> <sub>5,234</sub> .....	Yolk weight with percentage of protein, body weight, and intensity.....	+0.300±0.0342
<i>r</i> <sub>6,2345</sub> .....	Albumen weight with percentage of protein, body weight, intensity, and yolk weight.....	+0.388±0.0318

The values for *r*, the multiple correlation coefficient, indicate that percentage of protein in the diet, body weight, and intensity accounted for approximately 20 per cent of the variations present in egg weight. The remainder of the variation must be attributed to differences in quality of the protein supplements used, the inherent variability of the birds, and other causes unknown to the writers. The other coefficients may be interpreted in a similar manner.

#### EFFECT OF DIET ON HATCHABILITY

During each month of the present experiment, except May and August, eggs were incubated. It was possible, therefore, to measure the relationship between intensity of production throughout the year and hatchability, as well as between average intensity and average hatchability for each hen. Table 12 gives the zero-order correlations between hatchability and percentage of protein and intensity.

TABLE 12.—Zero-order correlations between hatchability, percentage of protein, and intensity

Factor No.	Egg-production factor	Correlation between factors indicated	
		2	3
1.....	Percentage of protein.....	+0.226±0.0347	-0.039±0.0365
2.....	Intensity.....		+0.165±0.0363
3.....	Hatchability.....		

The correlation between percentage of protein in the diet and intensity of egg production has been considered and found to be significant. The coefficient of correlation between percentage of protein in the diet and hatchability,  $r = -0.039 \pm 0.0365$ , is negative and

not significant. The coefficient of correlation between average intensity and hatchability,  $r = +0.165 \pm 0.0363$ , is very low but significant. The writers treated these zero-order correlations by the method of partial correlation. Percentage of protein correlated with intensity, with hatchability held constant, ( $r_{12.3}$ ) is not a logical relationship, for hatchability could not affect either of the other two factors. It became apparent that there is no significant relation between percentage of protein in the diet and average hatchability because the coefficient of net correlation,  $r = -0.083 \pm 0.0364$ , is not significant. The coefficient of net correlation between intensity and hatchability,  $r = +0.179 \pm 0.0363$ , is low but probably significant. It is likely that this latter correlation would be higher in a flock on a uniform diet or in a flock on diets containing successively larger percentages of the same protein concentrate, for it is shown that the effects of the several kinds of concentrate on hatchability bear no relation to their percentage of protein content.

If intensive laying depletes the hen's store of substances essential for the formation of hatchable eggs, or tends to do so, as Heuser (9) supposed, there should be a negative correlation between current production and hatchability. This does not necessarily require a negative correlation between average intensity and average hatchability, for a hen might alternate periods of very intense production with periods of broodiness or other nonlaying periods. She would thus have a low average intensity but high current intensity for the periods of actual egg production.

The hatchability of all individual clutches of eggs incubated in the present experiment was correlated with the percentage of production of the hens producing them for the month in which they were produced. The value for this correlation is  $r = +0.197 \pm 0.0154$ . This correlation is low but very significant, being more than 10 times its probable error. However, since the data used were obtained from incubations throughout the year, it might show only that the seasonal conditions conducive to high egg production furnish the most favorable climatic conditions for the collection, storage, and incubation of eggs, without necessarily indicating inherent differences in hatchability in the eggs themselves. To find out whether this was true, hatchability for March and for April was correlated with intensity of production for the same months. This correlation is  $r = +0.225 \pm 0.0274$ ; it is more than eight times its probable error and is significant. This shows that even on the low-protein diets on which the hatchability was fairly good and the average intensity low, hatchability was best for the eggs of the hens laying most heavily. The correlation between current production and hatchability for a flock on a uniform diet would probably be higher. The coefficient of correlation between current hatchability and current intensity for diet 8 for the entire period of the experiment is  $r = +0.298 \pm 0.0415$ . It seems safe to conclude that hens able to lay eggs at a high rate on a particular diet will, in general, produce a higher percentage of hatchable eggs than those able to lay only at a low rate on the same diet.

#### EFFECT OF DIET ON FERTILITY

Table 13 shows the effect of the diets on the fertility of the eggs produced and includes comparative data on the hatchability of fertile eggs. In fertility of eggs the greatest difference between diet 9

and any of the other diets,  $5.81 \pm 1.89$ , was found between diet 9 and diet 2. This is barely three times its probable error. Eggs from birds on diets 1, 2, and 8 were significantly higher in fertility than eggs from birds on diet 6. Table 13 shows that no diet consisting of feedstuffs of vegetable origin alone gave higher fertility than any diet containing an animal-protein concentrate, though the eggs from birds on diets 7 and 4 were practically equal in fertility.

TABLE 13.—Effect of the various diets on the fertility of eggs and on hatchability of fertile eggs produced

Diet. No.	Eggs set	Fertile eggs	Differences in fertility of eggs from birds on indicated diet and on diet 9	Differences in fertility of eggs from birds on indicated diet and on diet 6	Hatch-ability of fertile eggs	Differences in hatch-ability of eggs from birds on indicated diet and on diet 9	Differences in hatch-ability of eggs from birds on indicated diet and on diet 8	Hatch-ability of total eggs (deduction made for break-age, in-fertility, and mortal-ity)	Poten-tial chicks per hen per day	Poten-tial chicks per kilo-gram feed eaten
	Num-ber	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Number	Number
1	736	96.6 $\pm$ 0.528	+4.68 $\pm$ 2.14	+7.22 $\pm$ 1.63	67.0 $\pm$ 1.96	+2.0 $\pm$ 3.19	-6.1 $\pm$ 2.66	50.2	0.271	2.01
2	657	97.7 $\pm$ .544	+5.81 $\pm$ 2.14	+8.35 $\pm$ 1.69	72.1 $\pm$ 2.06	+7.1 $\pm$ 3.27	-1.0 $\pm$ 2.93	57.6	.295	2.16
3	648	94.2 $\pm$ 1.10	+2.27 $\pm$ 2.12	+4.81 $\pm$ 1.93	62.8 $\pm$ 2.08	-2.2 $\pm$ 3.68	-10.4 $\pm$ 2.75	45.9	.237	1.76
4	652	92.5 $\pm$ 1.55	+5.6 $\pm$ 2.39	+3.10 $\pm$ 2.23	73.4 $\pm$ 1.89	+8.4 $\pm$ 3.15	+3.3 $\pm$ 2.61	49.6	.228	1.77
5	546	89.4 $\pm$ 1.89	-2.49 $\pm$ 2.62	$\pm$ 0	71.6 $\pm$ 1.97	+6.6 $\pm$ 3.20	-1.6 $\pm$ 2.67	53.3	.213	1.68
6	669	89.4 $\pm$ 1.60	-2.54 $\pm$ 2.42		61.9 $\pm$ 2.32	-3.1 $\pm$ 3.43	-11.2 $\pm$ 2.94	40.5	.191	1.55
7	525	92.4 $\pm$ 1.23	+4.9 $\pm$ 2.15	+3.03 $\pm$ 2.02	56.6 $\pm$ 3.47	-8.4 $\pm$ 4.29	-16.5 $\pm$ 3.91	45.0	.196	1.54
8	827	95.0 $\pm$ .86	+3.10 $\pm$ 2.01	+5.64 $\pm$ 1.82	73.1 $\pm$ 1.80	+8.1 $\pm$ 3.10		59.0	.329	2.55
9	664	91.9 $\pm$ 1.81		+2.54 $\pm$ 2.42	65.0 $\pm$ 2.52		-8.1 $\pm$ 3.10	51.0	.220	1.85

None of the differences in hatchability of eggs from birds on diet 9 and those from birds on the other diets is significant, but the eggs from birds on diets 3, 6, and 7 show significantly lower hatchability than those from birds on diet 8. Diets 2, 4, 5, and 8 gave a distinctly superior hatchability of fertile eggs.

Table 13 shows that diets 1, 2, and 8 are more efficient for chick production than the others. Diet 7 was notably poor.

EFFECT OF DIET ON EMBRYONIC MORTALITY AND TIME OF DEATH

Figure 3 gives the average mortality of each day for all eggs from birds receiving vegetable diets as compared with that for eggs from birds receiving animal protein. The curve for embryos from hens on vegetable diets had a peak in mortality during the second week of incubation which was not present in the curve for embryos from hens receiving animal protein.

The condition described by Dunn (6, 7) and Landauer (16) as chondrodystrophy is shown by the embryo on the right in Figure 4. This embryo died at about the eleventh day of incubation; a normal embryo of that age is included for comparison. The chief points of difference are the shortened and recurved legs, the parrotlike beak, and the sparse down of the chondrodystrophic embryo. These embryos were very often edematous. In the present experiment most of them died during the second week of incubation but a few lived longer; none hatched. Table 14 gives the distribution of

chondrodystrophic embryos among the eggs from birds on the several diets.

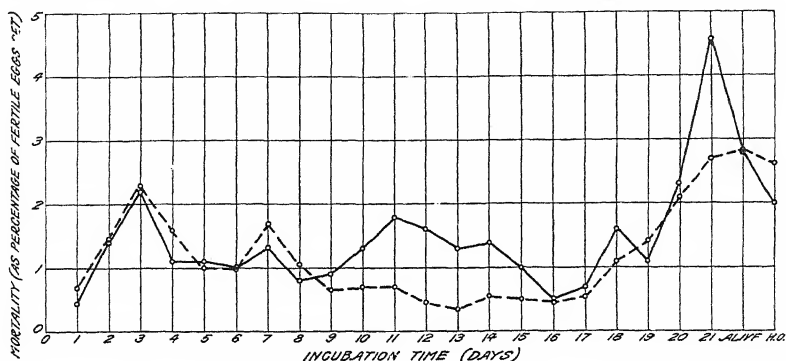


FIGURE 3.—Average percentage of mortality of embryos in eggs from hens on diets containing only vegetable feedstuffs (solid line) and in eggs from hens on diets containing animal-protein concentrate (broken line) as well. "H. O." = viable chicks helped out of shell on twenty-second day; "alive" = chicks alive in shell on twenty-second day

TABLE 14.—Number and percentage of chondrodystrophic embryos from eggs produced on the different diets

Diet No.	Chondrodystrophic embryos		Diet No.	Chondrodystrophic embryos		Diet No.	Chondrodystrophic embryos	
	Number	Percentage of fertile eggs set		Number	Percentage of fertile eggs set		Number	Percentage of fertile eggs set
1.....	4	0.56	4.....	2	.34	7.....	25	5.25
2.....	2	.31	5.....	7	1.40	8.....	3	.30
3.....	2	.33	6.....	30	5.03	9.....	16	2.61

Table 14 shows that the birds on diets containing animal-protein concentrates produced few chondrodystrophic embryos, whereas those on diets containing only feedstuffs of vegetable origin produced a relatively large number. The highest number from birds on a diet containing animal protein is 4, or 0.56 per cent of the number of fertile eggs set; the lowest number from birds on any diet consisting of vegetable feedstuffs is 7, or 1.4 per cent of the number of fertile eggs set. The eggs from birds on diets 6 and 7 had more than 5 per cent chondrodystrophic embryos. The average percentage of chondrodystrophic

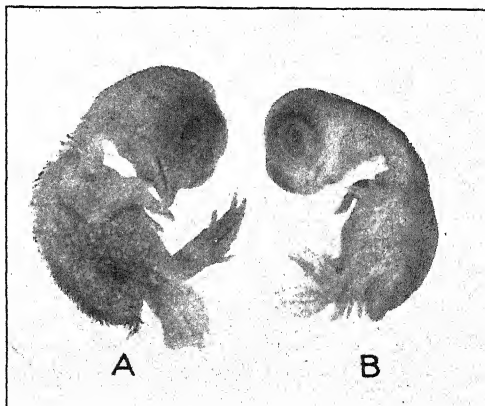


FIGURE 4.—Comparison of a chondrodystrophic embryo (B) with a normal embryo (A). Note the parrot-like beak and short, recurved legs of the former. Both embryos are about 11 days old

embryos in the eggs incubated from 34 hens which produced such embryos when fed vegetable diets was  $21.98 \pm 2.53$ , and from 12 hens fed the animal-protein diets the percentage was  $6.3 \pm 0.788$ . The difference between these averages is  $15.68 \pm 2.65$ ; the difference is six times its probable error and shows conclusively that the tendency toward chondrodystrophy was greater among the embryos from the hens on the vegetable diets. The closeness of the incidence of chondrodystrophy in the progeny of these hens on the vegetable diets to the classical 3:1 Mendelian ratio given by a mating of heterozygous individuals involving a single recessive character, is without significance since the cockerels used were rotated from pen to pen.

Dunn (7) and Hutt and Greenwood (10) found that the occurrence of chondrodystrophy among the progeny of hens was not according to chance. This was true in the present experiment, as shown in Table 15, which gives the frequency distribution of chondrodystrophy among the embryos from the hens on vegetable diets compared with the frequencies of a Poisson series. The fit is very poor. Dunn (7) considered that his failure to increase the incidence of chondrodystrophy by inbreeding ruled out his original hypothesis of inheritance, but Hutt and Greenwood (10) considered it probable that hens bear a gene or genes for susceptibility to chondrodystrophy, the action of which may be augmented by lack of sunlight.

TABLE 15.—Frequencies of chondrodystrophy among embryos from hens on vegetable diets, compared with frequencies of a Poisson series

Number of chondrodys- trophic embryos	Observed fre- quency	Theoretical frequencies of Poisson series, using $nq=0.5$ , <sup>a</sup> with average progeny	Theoretical frequencies with observed progenies	Number of chondrodys- trophic embryos	Observed fre- quency	Theoretical frequencies of Poisson series, using $nq=0.5$ , <sup>a</sup> with average progeny	Theoretical frequencies with observed progenies
0.....	121	94.012305	94.092440	4.....	2	0.244900	0.824135
1.....	16	47.006075	42.578035	5.....	2	.024490	.214675
2.....	6	11.751480	13.195460	6.....	2	.005115	.000465
3.....	6	1.958580	4.094790				

<sup>a</sup> The binomial is  $\left(\frac{27}{38} + \frac{1}{38}\right)^{14} \times 155$ . (The true value of the exponent, 14, is  $13.93 \pm 0.799$  = average sample progeny.)

Since the progenies varied in size, and the chances of occurrence of a particular number of chondrodystrophic embryos in a progeny are greatly affected by the size of the progeny, the values shown in the last column of Table 15 were calculated. The actual size of each progeny containing chondrodystrophic embryos was used in computing the probability of occurrence of the observed number of these embryos in that particular progeny. The average of the probabilities of all the progenies containing 0, 1, 2, 3, 4, 5, or 6 chondrodystrophic embryos was obtained and multiplied by 155, the total number of progenies, to obtain the values given in the table. These values give a somewhat better fit to the observed frequencies than do the frequencies obtained by using average progeny, but the fit is still too poor to permit the distribution to be attributed to chance.

According to the values in the third column of Table 15, a progeny of 121 embryos would be expected once in

31,000 times. Actually, it occurred twice in 155 times; one of the progenies contained 8, the other 11 embryos.

Table 16 gives the seasonal distribution of chondrodystrophy among the progenies of hens on the vegetable diets that produced one or more chondrodystrophic embryos at some time during the experiment. Those from hens on the diets containing animal protein are not included on account of their small number.

TABLE 16.—Seasonal distribution of chondrodystrophic embryos in eggs from hens on vegetable diets

Date of incubation	Fertile eggs set	Chondrodystrophic embryos			Date of incubation	Fertile eggs set	Chondrodystrophic embryos		
		Number	Fertile eggs	Average of eggs of individual hens that produced such embryos			Number	Fertile eggs	Average of eggs of individual hens that produced such embryos
	<i>Number</i>		<i>Per cent</i>	<i>Per cent</i>		<i>Number</i>		<i>Per cent</i>	<i>Per cent</i>
Oct. 2.....	12	1	8.3	5.0±3.37	Feb. 19.....	53	5	9.4	11.1±3.45
Oct. 30.....	31	6	19.7	32.6±9.99	Mar. 17.....	56	15	26.8	33.5±5.57
Nov. 27.....	30	2	6.7	5.6±2.58	Apr. 16.....	70	23	32.9	38.6±5.71
Dec. 23.....	46	6	13.0	17.2±5.61	June 16.....	70	16	22.9	25.8±5.02
Jan. 12.....	38	3	7.9	10.4±5.42	July 15.....	34	0	0	0

Treated in this way, the data indicate that the peak of incidence falls in October and in the period from March to June, inclusive. However, the probable error of the October percentage is so great that it is by no means certain that the high value obtained is other than a chance fluctuation. The data are not incompatible with the hypothesis that these diets were incapable of supplying a sufficient quantity of some substance in the eggs essential to the production of normal embryos and that this lack was accentuated by the small amount of ultra-violet rays received through the winter and spring, so that the peak of incidence was reached during the season of highest production. The peak in April corresponds with the peak in Dunn's (?) data, but not with that in the data of Hutt and Greenwood (10), which occurred in January. Opposed to the hypothesis that lack of ultra-violet rays influenced the incidence of chondrodystrophy in the present experiment is the fact that the diets contained 2 per cent of cod-liver oil of standard potency. It seems more probable that the high incidence of chondrodystrophy in the embryos from the birds on vegetable diets is due to a deficiency of some substance other than vitamin D. The seasonal distribution described by Dunn (?) and Hutt and Greenwood (10) might be accounted for in other ways. The winter diet of their birds was certainly more restricted than the summer diet, if their birds were permitted range. Deficiency in some vitamin other than D, present in certain protein concentrates, or qualitative deficiency in the protein itself seems more likely.

#### SUMMARY AND CONCLUSIONS

Single-Comb Rhode Island Red pullets and cockerels were fed diets containing various proteins of animal or vegetable origin to determine the effect of the proteins on egg production and hatchability. The

proteins comprised from 11.2 to 23.6 per cent of the diet. The basal diet consisted of a mixture of yellow corn meal, wheat bran, rolled oats, and alfalfa-leaf meal together with a mineral mixture and cod-liver oil. The various supplements to the diet were desiccated meat meal, crab-scrap meal, North Atlantic fish meal, dried buttermilk, dried-yeast preparation, soybean meal, cottonseed meal, and a combination of the meat meal, fish meal, and buttermilk.

The birds on the basal diet ate very well; they consumed practically as much feed as the birds on the supplemented diets. Current egg production was low; hatchability was fair.

Diet 1, meat-meal supplement, gave very good egg production and fair hatchability.

Diet 2, crab-scrap-meal supplement, was consumed readily and gave good egg production. The quantity of feed required to produce 1 g of egg was only a trifle higher than the quantity required when the birds received fish meal, and the hatchability of the eggs was better.

Diet 3, North Atlantic fish-meal supplement, gave good egg production, but the hatchability of the eggs produced was somewhat low.

Diet 4, buttermilk supplement, was excellent for winter egg production, egg weight, body weight, and hatchability of fertile eggs. Annual production was less than that of hens receiving diet 8, which was supplemented by meat meal, fish meal, and buttermilk.

The egg production on diet 5, yeast supplement, was not so good as that on the basal diet.

Diet 6, soybean-meal supplement, gave good egg production. Hatchability of fertile eggs was low, due in part to the fact that the incidence of chondrodystrophy in the eggs of certain hens was augmented.

Diet 7, cottonseed-meal supplement, delayed the beginning of egg production. It also increased the incidence of chondrodystrophy in the eggs of certain hens and gave low hatchability.

Diet 8, meat meal, fish meal, and buttermilk supplement, proved to be an excellent diet both for egg production and for hatchability.

Increasing the percentage of protein in the diet within the limits of 11.2 and 23.6 by the use of protein supplements of different origin augmented egg production (1) by increasing intensity of production; (2) by increasing egg weight through direct effect on yolk weight, and (3) indirectly, by increasing body weight and yolk weight, thereby increasing albumen weight.

Increasing protein level, within the limits stated, increased the quantity of egg produced per unit weight of feed eaten.

Increasing protein content decreased the efficiency of protein for egg production.

In general, during a given period, more eggs were hatchable from hens which laid intensely than from those which did not.

Diets containing proteins from vegetable sources only increased the incidence of chondrodystrophy in the embryos of hens likely to produce such embryos. Embryos in eggs from hens on such diets had a high second-week mortality.



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# THE INTERRELATIONS OF TWO HYMENOPTEROUS EGG PARASITES OF THE GIPSY MOTH, WITH NOTES ON THE LARVAL INSTARS OF EACH<sup>1</sup>

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## INTRODUCTION

Two imported hymenopterous egg parasites of the gipsy moth (*Porthetria dispar* L.) are established over a considerable part of the area of New England infested by that species, and it has been assumed that there was more or less competition between the two parasites when in the same area. It is the purpose of the writer to present some observations recorded during an attempt to determine the interrelationships of these parasites, *Anastatus disparis* Ruschka<sup>3</sup> and *Ooencyrtus kuvanae* (Howard),<sup>3</sup> when both occur in the same locality. In the course of the experiments some additional information concerning the larval instars of the parasites was obtained and is included in this paper.

*Anastatus disparis* (fig. 1) occurs in many parts of Europe and Japan. It was first colonized in this country in 1908 and since then has been liberated throughout most of the infested territory. *Ooencyrtus kuvanae* (fig. 2) is a native of Japan and was first colonized in this country in 1909. *A. disparis*, partly because of its wider distribution in the infested area of the New England States, is the more important parasite.

Previous publications dealing with the parasites of the gipsy moth contain accounts of these two species. Howard and Fiske<sup>4</sup> record instances of *Anastatus* larvae being parasitized by females of *Ooencyrtus* and of a generation of this parasite being carried through to maturity within the larvae of *Anastatus*. Crossman<sup>5</sup> states that in some instances larvae of both species have been found within the same host egg, but in such cases neither parasite has issued. It is also stated that it would seem from the observations made that *Anastatus* would suffer in an area where *Ooencyrtus* was abundant. Burgess and Crossman<sup>6</sup> state that these two parasites do not often conflict. These statements were suggested by observations made in connection with the importation, rearing, and colonization of these parasites, but the subject of the interrelations of the species has not previously been investigated in detail.

<sup>1</sup> Received for publication Mar. 28, 1932; issued February, 1933.

<sup>2</sup> The writer is indebted to C. W. Collins, in charge of gipsy moth and brown-tail moth investigations of the Bureau of Entomology, for his cooperation in making these experiments possible. Acknowledgment is also made to C. F. W. Muesebeck for suggestions incorporated in the plan of the experiments, and to various workers on the staff for information taken from notes on file in the laboratory.

<sup>3</sup> Order Hymenoptera, superfamily Chalcidoidea, family Encyrtidae.

<sup>4</sup> HOWARD, L. O., and FISKE, W. F. THE IMPORTATION INTO THE UNITED STATES OF THE PARASITES OF THE GIPSY MOTH AND THE BROWN-TAIL MOTH: A REPORT OF PROGRESS, WITH SOME CONSIDERATION OF PREVIOUS CONCURRENT EFFORTS OF THIS KIND. U. S. Dept. Agr., Bur. Ent. Bul. 91, 312 p., illus. 1911.

<sup>5</sup> CROSSMAN, S. S. TWO IMPORTED EGG PARASITES OF THE GIPSY MOTH, *ANASTATUS BIFASCIATUS* FONSCH and *SCHEDIUS KUVANAE* HOWARD. Jour. Agr. Research 30: 653. 1925.

<sup>6</sup> BURGESS, A. F., and CROSSMAN, S. S. IMPORTED INSECT ENEMIES OF THE GIPSY MOTH AND THE BROWN-TAIL MOTH. U. S. Dept. Agr. Tech. Bul. 86: 33. 1929.

## SEASONAL-HISTORY NOTES ON THE HOST AND PARASITES

The greater part of the life of the gipsy moth is spent in the egg stage. The eggs, in a compact mass covered by the hairs from the abdomen of the female, are deposited during July and early August on

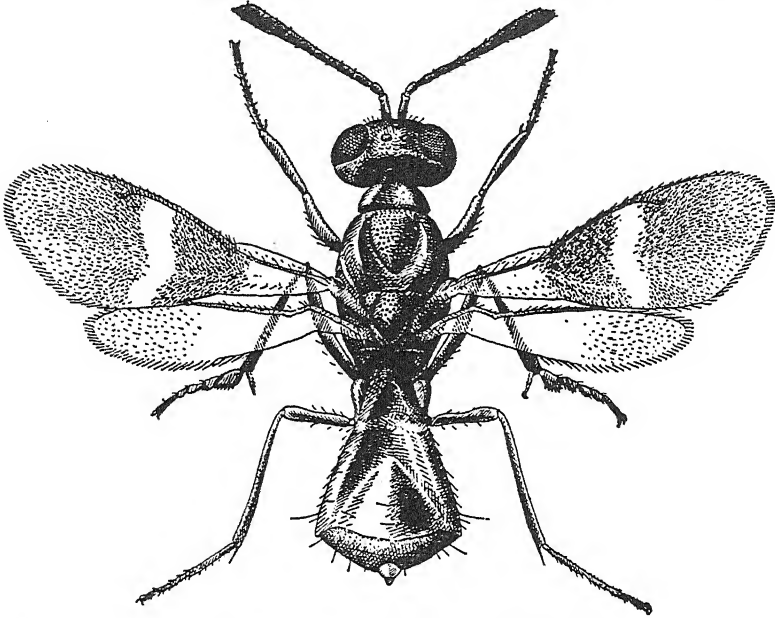


FIGURE 1.—*Anastatus disparis*: Adult female,  $\times 22$ . (Howard)

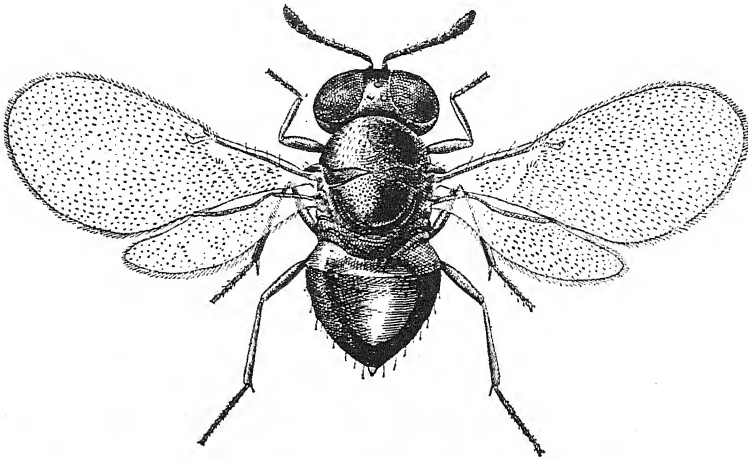


FIGURE 2.—*Ooencyrtus kuzanae*: Adult female,  $\times 35$ . (Howard)

tree trunks, branches, stones, and, in fact, on almost any object in the immediate vicinity of the point where the female moth, which is incapable of flight, has emerged. Hatching usually occurs during the first two weeks of the following May. The young caterpillars grow

rather rapidly, and the larval life is completed in early July, when pupation occurs. The duration of the pupal period is about 11 days for females and 14 days for males. Oviposition occurs directly after the fertilization of the female moth.

*Ooencyrtus kuvanae* usually hibernates in the forest débris as an adult female. The females that survive the winter appear during the first warm days of April and have been observed ovipositing during the latter part of that month on bright warm days. Six weeks is required for the first generation to develop in overwintering eggs of the gipsy moth, the adults appearing during the last week of May and early June. Those of the second generation emerge during the first half of July. About three weeks is required for the development of the third generation, which is the first that is able to develop entirely on the newly deposited eggs of the host, emergence of the adults occurring during the first half of August. Under laboratory conditions a generation develops in 15 days. There is some overlapping of generations. Part of the adults of the first and second generations oviposit in dead and infertile eggs in the early spring, whereas others live until the new eggs are deposited and attack these. There is sufficient time for four complete generations and a partial fifth in New England.

*Anastatus disparis* hibernates as a full-fed larva within the egg of the gipsy moth and is rather well protected by the coating of hairs covering the egg mass. The adults may emerge as early as the middle of June or as late as the middle of August, but the majority are present in the field at the time of maximum oviposition by the gipsy moth, which normally occurs in July. The period from egg deposition to larval maturity is from 12 to 15 days. In some years, however, there is a partial second generation, which is of slight importance.<sup>7</sup> From this résumé it is apparent that the life history of *Anastatus disparis* coincides more closely with that of its host than does that of *Ooencyrtus kuvanae*. Furthermore, the hibernation of *Anastatus* is more successful than that of *Ooencyrtus* in the New England States because of its ability to withstand lower temperatures. It has been very difficult to bring adults of *Ooencyrtus* through the period of hibernation in laboratory experiments. Observations in the field indicate that the number of females living through the winter is very small.

#### OUTLINE OF EXPERIMENTS

In an attempt to duplicate conditions as they might occur in the field, two parallel sets of experiments were conducted. In one set eggs were subjected first to *Anastatus disparis* and then to *Ooencyrtus kuvanae*; in the other they were subjected first to *O. kuvanae* and then to *A. disparis*. In the field no doubt there are more instances of *A. disparis* attacking the eggs first than of *O. kuvanae* attacking first, because of the greater numbers of *Anastatus* present at the time of egg deposition. An outline of the experiments conducted is given below.

(1) In experiment 1 a number of host eggs were subjected to parasitization by individual females of *Anastatus*. Each day a few eggs that had been attacked were placed with females of *Ooencyrtus*.

<sup>7</sup> The foregoing facts concerning the life histories of the host and parasites were taken from the previously cited papers of Burgess and Crossman, and of Crossman, with the exception of the duration of the pupal period of the host, which was taken from unpublished data collected by J. A. Millar of the gipsy-moth laboratory.

This was continued for 15 days to obtain a series of eggs containing *Anastatus* that had been attacked by *Ooencyrtus* at different periods of the egg and larval development of *Anastatus*.

(2) Experiment 2 was essentially like experiment 1, except that *Ooencyrtus* was allowed to attack the host eggs first, after which they were subjected to *Anastatus*. At the end of 15 days *Ooencyrtus* adults were issuing, which made it unnecessary to continue the experiments.

(3) One egg cluster from egg masses deposited by female moths on thin blocks of wood was fastened to the bottom of each of seven cages constructed of fine copper screening. These cages were cylindrical, 4 inches in depth and 5 inches in diameter, and had removable tin covers to which were fastened small metal handles; the cages themselves were fastened to wooden bases. Ten females of *Anastatus* were left in each cage for two days, after which all were removed. Ten females of *Ooencyrtus* were then introduced into one of the cages and left for two days. This was repeated with the other cages at 2-day intervals until all the cages had been treated in this manner. These cages and those used in experiments 4 and 5 were placed in the laboratory yard.

(4) Ten females of *Ooencyrtus* were placed in each of seven cages, of the foregoing type, and allowed to remain there for two days. The adults were then removed, and 10 females of *Anastatus* were placed in one of the cages and kept there for two days. The other cages were taken one at a time at 2-day intervals and treated in the same manner until all egg masses had been subjected to attack by both species.

(5) Five similar cages were used containing 10 females of each species caged together for 18 days.

(6) Two hundred and forty females of *Anastatus* were placed with 12 entire egg masses in a wooden box having a glass top. At the expiration of 2 days the *Anastatus* females were removed and 2 of the egg masses placed with 40 females of *Ooencyrtus*, where they were kept for 2 days. This was repeated at 2-day intervals with fresh parasites until all the masses had been subjected to *Ooencyrtus*.

(7) Experiment 7 was similar to experiment 6, except that *Ooencyrtus* was allowed to attack first, and the egg masses were then subjected to *Anastatus*.

(8) Experiment 8 was similar to experiment 6, except that the number of parasites used was decreased by one-half.

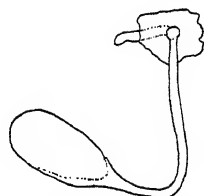
(9) Experiment 9 was similar to experiment 7 except that the number of parasites used was decreased by one-half.

This combination of experiments seemed comprehensive enough to give valuable information as to the behavior of each of the parasites in relation to the other. The first two experiments are the most valuable because the parasites were kept under observation at all times. The remainder of the experiments were conducted as a check upon the first two.

#### PROCEDURE FOLLOWED IN THE EXPERIMENTS

In the first experiment the following method was used: One gipsy-moth egg was fastened to a small piece of paper by means of shellac and placed in a small glass vial (35 by 10 mm) with 1 female of

*Anastatus*. This was done to prevent interference between the females, such as had been noticed when a number of eggs were confined with several females. One man could watch about 50 of these vials with comparative ease, for the adults work rather slowly. When an egg was observed to have been attacked, it was removed and examined very carefully for the presence of a puncture made by the ovipositor of the female. Such a puncture would not necessarily signify the presence of a parasite egg (fig. 3), for the females often punctured the host eggs merely to provide openings at which to feed. Accordingly, it was necessary to subject entire egg masses to *Anastatus*, for the coat of hairs decreased the feeding by the adults. After the masses had been exposed for one day they were broken up and the eggs cleaned by rubbing them over cheesecloth stretched tightly over a wooden frame. The punctured eggs were then picked out for use in this experiment. For the first four days after attack by *Anastatus* it was impossible to determine definitely whether parasitism had taken place, but from that time on no egg was used which was not definitely known to contain an *Anastatus* larva. A few of these eggs were offered to *Ooencyrtus* each day, and as soon as they were attacked they were removed and examined for the presence of the egg of *Ooencyrtus*. This egg (fig. 4) has a "stalk" which extends

FIGURE 3.—Egg of *Anastatus disparis*,  $\times 110$ . (Howard and Fiske)FIGURE 4.—Egg of *Ooencyrtus kuvanae*,  $\times 140$ 

through the host egg sufficiently far to be easily recognized with a binocular microscope. This stalk, which is merely the end of the petiole of the egg, is definite evidence that *Ooencyrtus* has deposited an egg. By this method a series of host eggs was obtained containing eggs or larvae of *Anastatus* and which had then been subjected to *Ooencyrtus* while the former was in different stages of development.

The second experiment, while essentially like the first, differed slightly in method. Individual eggs were subjected to attack by *Ooencyrtus* and then submitted to *Anastatus*. It would have been better if entire egg masses had been submitted to *Ooencyrtus* and then to *Anastatus* and those eggs picked out that showed evidence of attack by both species. However, such a procedure would have involved sifting out and looking over so many eggs that it was impractical, and as *Ooencyrtus* readily attacked individual eggs no entire masses were used.

In all the remaining experiments the following method was employed: After the egg masses mentioned in the outline of work had been treated and the adults of *Ooencyrtus* had issued, the eggs were sifted from the clusters and counted. They were then separated into three groups—eggs from which *Ooencyrtus* adults had issued, eggs containing larvae of *Anastatus*, and unparasitized eggs. The adults of *Ooencyrtus* which emerged from these were not counted, for in many

cases two adults issue from one host egg. The eggs containing *Anastatus* larvae were examined for evidence of *Ooencyrtus*, as in the previous experiments.

### RESULTS OF THE EXPERIMENTS

An analysis of the information obtained from these experiments must take into account the direct observations made on the habits of the parasites as well as the results expressed in tabular form. If this is done the results of the experiments can be explained more clearly than would be possible if only the tabulations were used.

The results obtained in experiment 1 are shown in Table 1. A study of Table 1 reveals several significant facts. The more striking perhaps is that the only *Ooencyrtus* that reached maturity in the entire experiment were found in the eggs that were attacked during the period from a few hours to four days after attack by *Anastatus*. It was during this period that host eggs were used which were not definitely known to contain *Anastatus*, and it is doubtful whether any of the emerging *Ooencyrtus* developed within an egg that contained *Anastatus*.

TABLE 1.—Results obtained from gipsy-moth eggs that were attacked first by *Anastatus disparis*, then subjected to *Ooencyrtus kuvanae*

Period between attack by <i>Anastatus</i> and attack by <i>Ooencyrtus</i> .....days.....	Less than 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Eggs showing evidence of attack by both species.....number.....	10	10	9	11	17	9	10	4	4	0	0	0	0	0	0	0
Eggs in which <i>Anastatus</i> developed to maturity.....number.....	4	8	7	7	8	9	10	4	4	5	0	4	5	0	3	4
Eggs in which <i>Ooencyrtus</i> developed to maturity.....number.....	6	2	2	4	9	0	0	0	0	0	0	0	0	0	0	0

Another significant fact brought out by Table 1 is that the females of *Ooencyrtus kuvanae* were able to oviposit within an egg containing *Anastatus disparis* until it had reached the eighth day of its development. In some instances *Ooencyrtus* developed to the third instar, but when the *Anastatus* larva reached the last instar the only evidence of *Ooencyrtus* was the remnants of an incomplete anal shield. In other instances, owing to the irregularity of development, the *Ooencyrtus* females were unable to oviposit after six days. Observations showed this to be due to the mechanical action of the *Anastatus* larva. The larva is disturbed by the drilling operations of the *Ooencyrtus* female, and by the time the ovipositor enters the egg *Anastatus* is out of reach. The movements of the larva bring its head close to the opening made in the egg by the ovipositor of *Ooencyrtus*, and this places its body in a position where it can not be reached. Often the larva of *Anastatus* touched the ovipositor, which caused the female *Ooencyrtus* to leave the egg. A similar attempt was observed when *Anastatus* tried to oviposit in an egg containing *Ooencyrtus*, but with much less success on the part of *Ooencyrtus*, as the larva was somewhat handicapped by its attachment to the remains of its own eggshell, which restricted its movements. The ability of a larva of one parasite to move away from the ovipositor of another is due in part to the cautious manner in which the adult parasite feels



around within an egg before ovipositing, and also to the length of time required for a female to puncture the chorion of the egg.

From the results of this experiment it appears that *Anastatus* would suffer little in competition with *Ooencyrtus*. The presence of *Ooencyrtus* in the first part of the series is not surprising for the reason that it is impossible to make certain that host eggs contain *Anastatus* until five days after attack by this species. Aside from this short period the evidence is conclusive that *Ooencyrtus* does not displace the *Anastatus* larva.

The results of experiment 2 were rather disappointing, owing to the presence of a factor that could not be controlled. This was the feeding habit of *Anastatus*, previously mentioned. It rather upset the results of the experiment and made it difficult to draw satisfactory conclusions. By making due allowance for this habit, however, some information of value may be derived from this experiment. (Table 2.)

TABLE 2.—Results obtained from gipsy-moth eggs that were attacked first by *Ooencyrtus kuvanae*, then subjected to *Anastatus disparis*

Period between attack by <i>Ooencyrtus</i> and attack by <i>Anastatus</i> .....days..	Less than 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	* 15
Eggs showing evidence of attack by both species.....number..	9	11	10	10	10	10	8	9	15	22	12	14	15	5	6	10
Eggs in which <i>Ooencyrtus</i> developed to maturity.....number..	7	7	10	10	9	4	2	4	5	8	7	10	11	1	1	8
Eggs in which <i>Anastatus</i> developed to maturity.....number..	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eggs containing both species.....number..	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0
<i>Ooencyrtus</i> considered as killed by feeding of <i>Anastatus</i> adult.....number..	0	1	0	0	1	5	6	4	10	13	5	4	4	4	5	2

\* At this time *Ooencyrtus* adults were issuing from host eggs.

Two facts stand out in Table 2: (1) The very small number of *Anastatus* that matured and (2) the large number of *Ooencyrtus* whose deaths were attributed to the feeding of the *Anastatus* females. Both of these circumstances resulted from the feeding habits of *Anastatus*, for it was noted throughout the experiment that the majority of the *Anastatus* females spent their time puncturing the host eggs in order to feed and in many cases did not oviposit. The death of a larva of *Ooencyrtus* noted in the third column of Table 2 was not caused by direct feeding by *Anastatus* but resulted from a reduction of available food, which prevented the larva from completing its growth. The later deaths, however, were caused by direct feeding of the adult *Anastatus* upon the body substance of the larvae or pupae of *Ooencyrtus*. In some instances it was possible to see the feeding tube constructed by the parasite extending from the body of the larva or pupa to the surface of the egg.

In all, four normal *Anastatus* larvae matured in this experiment. These were found in the eggs parasitized by *Anastatus* one day or less after *Ooencyrtus* had oviposited in them. In contrast with the condition in the first experiment it is certain that every egg used in this experiment had been oviposited in by *Ooencyrtus* before it was exposed to *Anastatus*. Therefore, in these four cases the egg or early-instar larva of *Ooencyrtus* must have been destroyed by the develop-

ing *Anastatus* larva. The remaining five *Anastatus* larvae occurring in this experiment were found in company with *Ooencyrtus*. In three instances the *Ooencyrtus* were larvae, and in the last instance there were two *Ooencyrtus* pupae within the one host egg with two larvae of *Anastatus*. In the instances where the egg contained both species all the *Ooencyrtus* larvae and pupae and four of the five *Anastatus* larvae were dead, the remaining one being barely alive.

The results in Table 2 indicate that it is possible for *Anastatus* to oviposit in an egg containing *Ooencyrtus* in any of its developmental stages, as some of the *Ooencyrtus* had pupated in nine days. There is some doubt whether either species would be able to complete its development if the attack by *Anastatus* occurred after the *Ooencyrtus* larva had reached the second instar, for in the eggs used in this experiment the second instar was reached on the third day, and no *Anastatus* matured in eggs attacked after two days had elapsed.

The importance of the feeding of the females of *Anastatus* would be over-emphasized if the figures in Table 2 were taken as an indication of the extent of its feeding under field conditions. The opportunity for the females to feed is much reduced on an entire egg mass because of the coating of hairs that covers a normal egg cluster. Egg masses will occasionally be found that are only partly covered, and in such cases considerable feeding by *Anastatus* might occur. If feeding did occur on the newly deposited eggs the development of the embryo would be prevented, as has been observed in some instances in the laboratory.

It is probable that experiment 1, in which the eggs were first attacked by *Anastatus disparis* and then by *Ooencyrtus kuvanae*, represents the situation more nearly as it occurs under field conditions, for it is probable that *A. disparis* is present in greater numbers at the time of deposition of gipsy-moth eggs than is *O. kuvanae*.

As already explained, the remaining experiments listed in the plan were conducted in order to check the results of the series in which individual eggs were used. As a whole the experiments were somewhat disappointing because the parasitism by *Anastatus* was lower than was expected. The adults did not work well in captivity under any conditions. The results, however, compare very well with those of the first two experiments. There is no indication of serious conflict between the two species, but it did appear that in a few instances *Ooencyrtus* oviposited in an egg containing *Anastatus* which resulted, as in the first experiment, in no development of *Ooencyrtus*. The results of these later experiments are not shown in tabular form because they present no information in addition to that furnished by the first two experiments.

Special efforts were made to duplicate an example, previously mentioned, of *Ooencyrtus* acting as an internal parasite of hibernating *Anastatus* larvae. Many attempts have been made in previous years to observe an incident of this sort, but without success. The writer, during these experiments, succeeded in obtaining reproduction of *Ooencyrtus* upon hibernating larvae of *Anastatus* by artificial means. Host eggs containing *Anastatus* larvae were placed for periods of from 4 to 15 minutes in a vial containing cotton that had been dipped in chloroform. These eggs were then subjected to attack by *Ooencyrtus* females, and oviposition was observed in some instances. The *Anastatus* larvae recovered from the effects of the

chloroform, but those larvae that had been attacked were killed by the developing *Ooencyrtus* larvae. It is possible that isolated instances of hyperparasitism occur, but only on larvae of *Anastatus* that are dead or dying at the time of attack by the females of *Ooencyrtus*.

#### INTERPRETATION OF THE RESULTS

The results of the experiments here recorded indicate that it is advantageous to have both *Anastatus disparis* and *Ooencyrtus kuvanae* in the same locality. Theoretically it is possible that conditions might arise under which *Ooencyrtus* would be unable to reproduce itself, as, for instance, if *Anastatus* adults were present in such numbers as to utilize all the available host eggs first. If, on the other hand, *Ooencyrtus* had parasitized all the available eggs, it would be difficult for *Anastatus* to reproduce.

It is impossible, within reasonable limits, to ascertain the maximum percentage of host eggs that the varied sizes and shapes of the egg clusters make available to these parasites. Competition between the parasites would occur if the combined percentages of parasitism approached the limit of available host eggs. Field studies in the areas of New England where both these species are found do not indicate that there is any such conflict.

*Anastatus disparis* appears to be better suited than *Ooencyrtus kuvanae* to its host and to New England because its seasonal history parallels that of its host and because its hibernation is more successful in this area. *Anastatus* does not suffer from competition with *Ooencyrtus*, which succeeds in producing some parasitism in addition to that caused by *Anastatus*. The control exerted by the combined parasitism of the two species is greater than that which would be effected by either parasite working alone.

#### LARVAL INSTARS OF THE PARASITES

The literature reviewed by the writer mentions only three larval instars for each of these two parasites. In the course of the investigations recorded in this paper five larval instars were observed in each species. Accordingly, the following descriptions are presented, and the mandibles of the five instars are figured.

#### LARVAL INSTARS OF *OOENCYRTUS KUVANAE*

The first-instar larva of *Ooencyrtus kuvanae* is a small white maggot which, after hatching, is partly enveloped posteriorly with the remains of the eggshell. The body of the larva, indistinctly segmented beyond the fifth segment, tapers anteriorly from the fourth segment to the hemispherical head and posteriorly to the point of attachment to the hatched eggshell, where two open spiracles may be seen. After each molt the cast skin passes back to the posterior end of the larva and is retained, forming a shield covering the anal portion of the larva. The subsequent instars produce no great changes in the appearance of the larva except for the relatively shorter and stouter appearance it takes on with continued feeding. Just before pupation the last-instar larva becomes freed from the anal shield, which usually remains pressed against the inner surface of the eggshell of the host. There is no difficulty in distinguishing the larvae of either of these species on the basis of mandibles alone, but a simple means of identification of *Ooencyrtus* is the presence of the anal shield.

Figure 5 illustrates the differences between the larval mandibles of *Ooencyrtus* in the various instars.<sup>8</sup> In many instances it was possible to obtain the last four pairs of mandibles from one individual, but it was difficult to locate the mandibles of the first instar. The passage of the cast skins toward the posterior end of the larva takes place rather slowly, so it was possible to locate the mandibles of the second, third, and fourth instars in order on the body of the larva from the anterior end of the anal shield toward the head of the larva, which contained the last-instar mandibles. The first-instar mandibles were obtained from freshly hatched material.

The first-instar mandibles are rather peculiar in shape. It is impossible to determine with a compound microscope the limits of the bases of the mandibles because they are colorless and exceedingly small, measuring but 0.0038 mm in length. The points of the mandibles turn away from each other, differing in this respect from those of the succeeding instars. The average length of the mandibles measured for each instar was 0.0138 mm for the second instar, 0.0207 mm for the third, 0.0276 mm for the fourth, and 0.0483 mm for the last. There is a gradual transition from the second-instar mandible, which presents an acute angle between the tooth and the base, to the last-instar mandible with its broad sweeping inner margin. From colorless first-instar and second-instar mandibles the change is gradual to a deep yellow last-instar mandible.

#### LARVAL INSTARS OF *ANASTATUS DISPARIS*

The first-instar larva of *Anastatus* is whitish and cylindrical, with a chitinized thimble-shaped head and a 13-segmented body which is broadest at the fourth or fifth segments and terminates in a bifurcate appendage. The body is rather densely clothed with spines and long sensory setae which are more numerous on the ventral portions. In the second instar the larva changes greatly in appearance. It loses the tegumentary spines and the bifurcate appendage, the head becomes more nearly spherical in outline, and the body appears relatively shorter and stouter. There is little change in the appearance of the larva from this instar through the following instars except for the continued thickening of the body, which in the last instar gives the larva a grublike appearance.

The larval mandibles of *Anastatus* (fig. 6) are much larger and heavier than those of *Ooencyrtus*. The mandibles of the first-instar larva, 0.0155 mm in length, are comma shaped, black at the distal end, becoming gradually lighter toward the base. The mandibles of the second-instar larva measure 0.0186 mm in length, those of the third instar 0.0276 mm, those of the fourth 0.0379 mm, and those of the hibernating or last-instar larva 0.0583 mm. The mandibles of the second-instar larva are colorless, whereas those of the third instar take on a light-brown color, which is confined mainly to the distal portion. The color of the mandibles increases in depth in the remaining instars, becoming a conspicuous reddish brown in the hibernating larva.

<sup>8</sup> The drawings of the mandibles of both species were made with the cover glass pressed down on a thin mount until all portions of the mandible, as far as possible, were in the same plane. All measurements were made in a straight line from the point of the mandible to the most distant visible point of the base.

## SUMMARY

In a large part of the New England area infested by the gipsy moth two imported hymenopterous parasites of the gipsy moth have become established. The first, *Anastatus disparis* Ruschka, is common in Europe and Japan, but the second, *Ooencyrtus kuvanae* (Howard), before its introduction into the United States was found only in Japan. From early observations made upon these parasites, it was suspected that there might be serious competition between them when present in the same locality. Since no attempts had been made to ascertain the interrelationships of these parasites, the writer conducted a series of experiments to determine the behavior of the two under conditions that would demonstrate competition if any were present. These

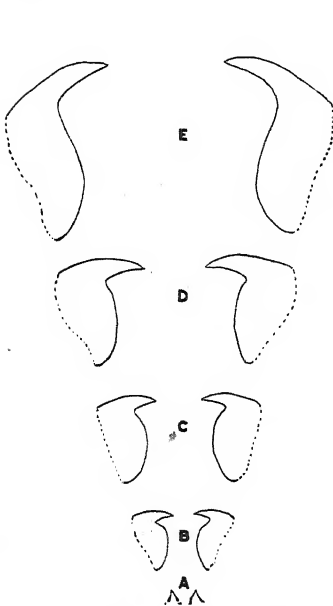


FIGURE 5.—Larval mandibles of *Ooencyrtus kuvanae*: A, B, C, D, E, First, second, third, fourth, and fifth instars, respectively

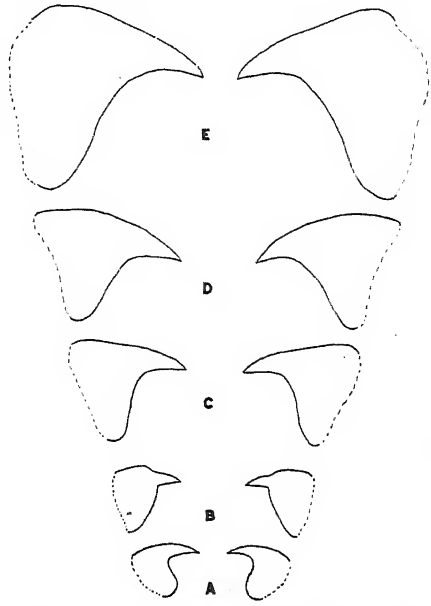


FIGURE 6.—Larval mandibles of *Anastatus disparis*: A, B, C, D, E, First, second, third, fourth, and fifth instars, respectively

experiments represented conditions as they occur in nature, that is, host eggs subjected to attack by either species were available for attack by the other at all stages in the development of the parasite which first attacks. Because *Anastatus* adults are probably more numerous at the time of deposition of host eggs, the greater number of instances of competition would be those in which *Anastatus* attacked first and *Ooencyrtus* attempted to reproduce upon those eggs already occupied by *Anastatus* in the egg or larval stages.

The experiments showed that there is little serious competition between the two parasites. Although *Ooencyrtus* females can attack a host egg containing *Anastatus* from the time that *Anastatus* is in the egg stage until it has become an early third-instar larva, it appears that *Anastatus* does not suffer from the attack. An egg containing *Ooencyrtus* throughout its egg, larval, and pupal life is to some extent

susceptible to attack by *Anastatus*, but except for successful oviposition while *Ooencyrtus* is in the egg stage, which results in *Anastatus* developing, neither of the parasites succeeds in completing development. Since there is little conflict between the parasites in the same area under the conditions simulated in these experiments, the presence of the two may be considered advantageous, for parasitism by *Ooencyrtus* affords additional control to that effected by *Anastatus*. *Anastatus*, however, is the preferred parasite (1) because it is a single-generation parasite having a life history which coincides very closely with that of the host itself and (2) because it hibernates successfully in the area of New England infested by the gipsy moth. *Ooencyrtus* is multiple brooded and depends largely upon infertile and killed host eggs for the early spring generation. It hibernates as an adult, and suffers great losses during the winter months.

In the course of the investigations it was noted that, contrary to the general belief, there are five rather than three larval instars in the case of both the parasites. Short descriptions of the larvae are given, and the larval mandibles of both parasites are illustrated.

# CATALASE ACTIVITY AND RESPIRATION IN THE LEAVES OF GROWING BARLEY<sup>1</sup>

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## INTRODUCTION

There has been some difference of opinion as to whether there is an association between catalase activity and respiration. In 1910 Appleman (1)<sup>2</sup> noted a correlation between the two in the potato tuber and later (2) observed the same thing in potato tubers manipulated in various ways to modify respiration and in stem and seed ends of the same tuber. He found a similar correlation in stored sweet corn (3), and concluded that catalase activity is a fair index of the comparative intensity of respiration in the tissues of sweet corn and potato tubers. Morinaga (11), from studies of rice seedlings grown under differing conditions of aeration, concluded that there is a close positive connection between the two phenomena, and Burge (4) and Burge and Burge (5) are convinced, from studies of a number of organisms, that catalase activity is definitely associated with metabolism and with respiration in particular.

Others, however, have not obtained such convincing results. Crocker and Harrington (6) found a positive correlation between respiration and catalase activity in seeds of Johnson grass (*Sorghum halepensis* (L.) Pers.) and probably in those of *Avena fatua* L. but not in *Amaranthus* seeds. Rhine (13), working with germinating seeds, concluded that catalase activity is a measure of metabolism only when there is no rapid change in respiration. Lantz (10) did not find a close correlation between catalase activity and respiration in germinating corn of different chemical composition (high and low oil, high and low protein).

The writer has demonstrated widely different degrees of catalase activity in different parts of the same barley (*Hordeum distichon*) plant.<sup>3</sup> The relationship between catalase activity and respiration in leaves of different age is shown in this paper.

## MATERIALS AND METHODS

Plants of pure-line Hannchen barley, C. I.<sup>4</sup> 531, growing in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.), were tagged for identification. A daily growth record of each leaf, based on measurements from the soil surface to the leaf tips, was kept for several days before the experiment. For each experiment three plants were selected, the homologous leaves of

<sup>1</sup> Received for publication Mar. 25, 1932; issued February, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 40.

<sup>3</sup> Unpublished data.

<sup>4</sup> C. I. denotes accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

which had made nearly equal growth. In these plants the developing spikes were small and still inclosed in the twelfth or boot leaf. From the first plant the six youngest leaves were removed, quickly weighed, and dried to constant weight at 80° C. at a pressure of approximately 6 cm of mercury. The second plant was laid away in a moist towel until the respiration run was started, when its leaves were individually tested for catalase activity. The leaves of the third plant were used for measuring the rate of respiration.

The respiration chamber was an Erlenmeyer flask of 125 c c capacity fitted with a 2-hole rubber stopper through which two glass tubes were passed, the upper ends projecting about 2 cm above the stopper. One of the tubes extended to within 2 cm of the bottom of the flask and the other only through the stopper. To the external ends of these glass tubes were fitted short pieces of rubber tubing with pinchcocks. The leaf to be investigated was loosely rolled on the finger, tied with a small cord, and suspended in the flask by slipping the end of the cord under a rubber band around the long glass tube. The chambers were set up in series with alternating blanks. The train was swept free of carbon dioxide, and 10 c c of nearly saturated barium hydroxide was introduced into each chamber through the hole stoppered by the short tube. The flasks were then shaken gently in a rocker shaker in the dark. In experiment 1, shaking was continued for 21 hours, and in experiment 2, for 23 hours. Temperatures ranged from about 24° to about 27° C. Blanks and respiration chambers were titrated alternately through the hole in the stopper, due precaution being used to prevent admission of atmospheric carbon dioxide. Titrations were made with 0.1362 normal hydrochloric acid, with phenolphthalein as an indicator. After the respiration run, the leaves were dried to constant weight and respiration rates were determined on both wet and dry weight bases. The slight error introduced by exposure to light and consequent assimilation during titration could not be determined.

Catalase determinations were made on leaves of the second plant by using the apparatus previously reported (12). Each leaf was triturated to a thin paste with washed sand, powdered calcium carbonate, and a small quantity of water. Enough water was added to make a 1 : 50 dilution based upon the green weight of leaf tissue. Two cubic centimeters of the suspension (equivalent to 0.04 g of green tissue) was then shaken with 3 c c of hydrogen peroxide in experiment 1 and with 2 c c in experiment 2. The volume of oxygen liberated for a dry-matter content of 0.008 g was calculated by using the average percentage of dry matter in homologous leaves of the other two plants.

#### EXPERIMENTAL DATA

The respiration rates which are based on dry matter in the leaves after the run probably are somewhat high. (Table 1.) Homologous leaves were reasonably similar in growth rate, amount of dead tip tissue, and size of sheath and blade, and the data should be comparable.

The youngest leaf gave off carbon dioxide most rapidly. The quantity given off by the next older leaf was much smaller and dropped much more gradually to the fourth youngest. Carbon dioxide



evolution possibly was due in part to oxidation of dead tissues in the two oldest leaves. This factor may account for variability in that part of the curves.

TABLE 1.—*Respiration and catalase activity in homologous leaves from similar plants of Hannchen barley*

EXPERIMENT 1				
Leaf No.	Respiration as CO <sub>2</sub> given off per hour per gram of—		Catalase activity as O <sub>2</sub> evolved in 5 minutes—	
	Green weight	Dry weight	For 0.04 g green tissue	For 0.008 g dry tissue
	<i>Mg</i>	<i>Mg</i>	<i>Cc</i>	<i>Cc</i>
10. ....	0.446	4.108	4.89	9.31
9. ....	.302	3.036	7.24	14.51
8. ....	.294	2.801	8.26	14.75
7. ....	.262	2.627	7.42	13.26
6. ....	.227	2.683	6.77	13.35
5. ....	.264	2.585	4.51	7.82
EXPERIMENT 2				
12. ....	0.584	5.043	5.37	8.92
11. ....	.338	2.873	11.74	18.67
10. ....	.309	2.403	14.80	22.81
9. ....	.283	2.266	13.22	21.45
8. ....	.285	2.587	12.27	21.15
7. ....	.243	2.471	10.52	19.77

In both experiments catalase activity was low in the youngest, most rapidly elongating leaf, but was much greater in the second youngest, which was elongating at about one-third the rate of the youngest, and was still greater in the next older. During senescence—indicated by greater length of dead leaf tip—catalase activity dropped off rapidly. Figure 1 gives a direct comparison between the respiration and catalase activity of homologous leaves on the two pairs of plants.

#### DISCUSSION

Hover and Gustafson (8, p. 39) state that "as the leaves of corn, sorghum, wheat, and oats increase in age there is a decrease in rate of respiration; but that as the leaves become still older (past about middle age) the rate gradually increases." Their data, however, show this conclusion to be fully justified only in the case of sorghum. In their experiments the oldest corn leaves at time of pollination showed a higher rate of respiration than the youngest; in oats both plants used showed a rise with age, and in one wheat plant the youngest leaf showed a lower rate than did the next older, whereas in the other wheat plant used the situation was reversed.

In the present experiments it is assumed that the quantity of carbon dioxide given off is a measure of the respiration of the living tissues in the plant part. The results show a relatively high respiration rate for the youngest leaf of barley. There is a sharp decline in the curve to the next older leaf, after which the decrease in successively older leaves is much less, and at the fourth youngest leaf an approximate level is reached. This leaf had finished growth in length a week or

more before respiration was determined and had died back about 2 cm at the tip. Leaves still older had still more dead tissue, and the shape and irregularities of the respiration curve probably can be explained

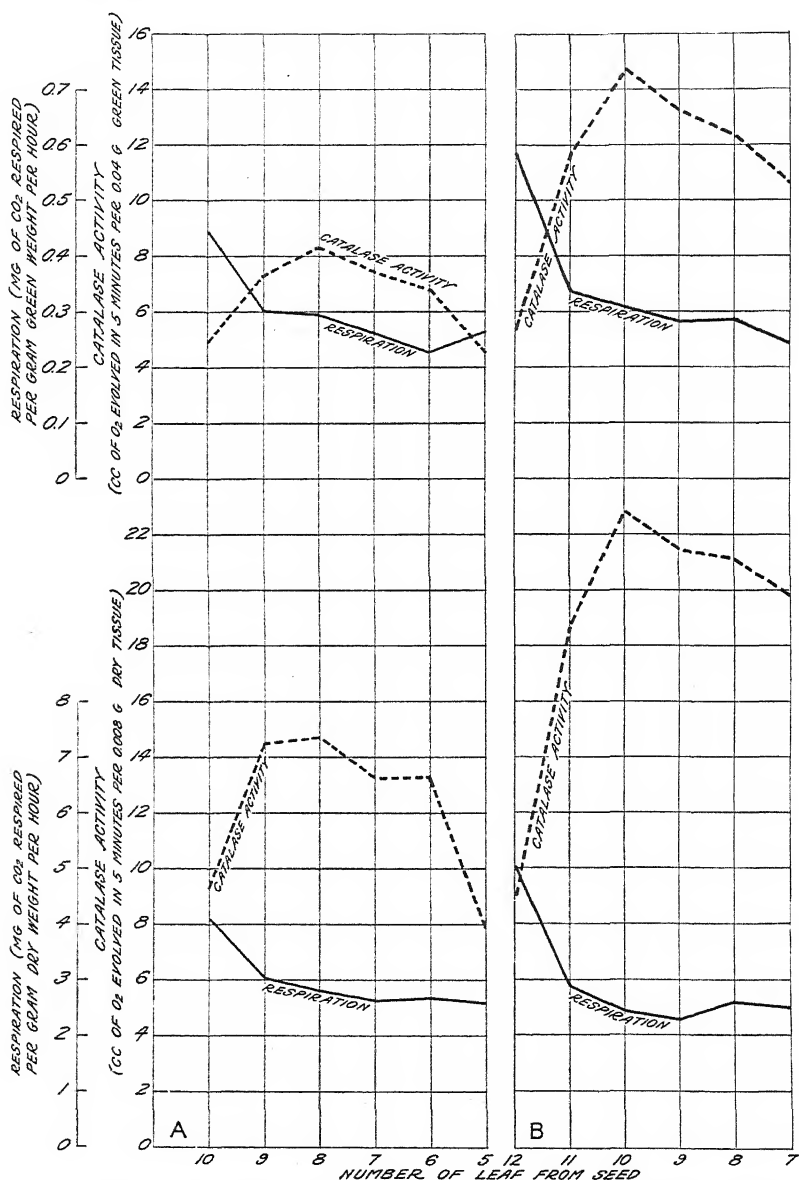


FIGURE 1.—Respiration and catalase activity in homologous leaves from similar plants of Hannechen barley: A, Experiment 1; B, experiment 2

in part by the accumulation as age progresses of nonrespiring structural and senescent tissues. Rate of respiration, highest in the young leaf, decreased rapidly with advancing age to a point where it probably remained constant on the basis of living tissue.

Knott (9) found the youngest and oldest leaves of spinach and celery usually low in catalase activity as compared with those of intermediate age. Ezell and Crist (7) found a significantly negative correlation between catalase activity and plant size in lettuce but not in radish or in spinach.

In the experiments herein described catalase activity was relatively low in the youngest leaf of barley but increased in successively older leaves up to maturity. It then fell off rapidly with increasing necrosis of the oldest leaves.

On comparing catalase activity and respiration (fig. 1) a negative correlation is found between the two phenomena for the three youngest leaves. In the older leaves the curves are irregular and the relationships less definite, since catalase activity appears to decrease as living material decreases in the tissues, and apparent respiration possibly includes the production of carbon dioxide by oxidation of dead tissues.

Since in these experiments the only correlation discernible is negative, and since positive correlations have been found in stored potato tubers and sweet corn (1, 2, 3), in germinating seeds (6, 14), and in rice seedlings (6), it seems highly probable that in no case is the relationship causal.

The nature of these results, contrary to those of most investigators, raises the question of the significance of catalase activity. The writer has assumed that the total catalase activity of the sample can be measured by prescribed methods. This assumption is true only if the agency responsible is a by-product of metabolism or if it is some part of the metabolic machinery which remains relatively stable and is not used up in the process. If either of these hypotheses is correct it must be concluded from the contradictory nature of the experimental data available from this and other studies that there is no causal relation between respiration and catalase activity.

On the other hand, the definition of catalase or catalase activity includes nothing but its reaction with hydrogen peroxide, which is the sole basis of its detection and determination. If this reaction occurs in normal tissues it is impossible to make an accurate determination of the agent producing it by shaking a tissue suspension with hydrogen peroxide, since the results then will be produced by the unused portion and will indicate only the excess of catalase manufactured over that utilized. It is conceivable that respiration and total catalase produced are positively correlated. In that case, in order to explain the results of these experiments it would be necessary to assume that in barley leaves high respiration is associated with a proportionately greater consumption of the catalase-activating agency, leaving a determinable surplus actually smaller in amount than when respiration is low.

#### SUMMARY

The rate of respiration was high in young barley leaves and decreased in proportion to the rapidity with which the leaves matured.

Catalase activity was low in young barley leaves and increased to a maximum at about the time of leaf maturity, after which it decreased with decrease of living material in the tissues.

A negative correlation was found between respiration rate and catalase activity in homologous younger leaves of comparable barley

plants. In older leaves relationships were indefinite, probably masked by increasing amounts of structural material and necrotic tissues.

Since, contrary to the results of other investigators, a negative correlation was found between catalase activity and respiration, it is highly probable that any correlation is fortuitous.

Current methods of determination probably can measure total catalase activity only if that phenomenon is a by-product of metabolism or has a function in the tissues other than the breaking down of hydrogen peroxide.

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# A METHOD FOR DETERMINING THE QUANTITY OF MINERAL OIL RETAINED BY LEAF SURFACES AFTER SPRAYING<sup>1</sup>

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## INTRODUCTION

Perhaps the most important factor to be considered in the study of oil emulsions as insecticides is the amount of oil deposited upon foliage and insects. The quantity of oil deposited on a unit area of leaf surface depends upon the concentration of oil in the emulsion, the hardness of the water used for dilution, the concentration of the emulsifier, the method of application, the time of spraying, and many uncontrollable factors which only roughly regulate the effectiveness of the preparation. Hitherto no accurate figures have been available which would indicate what amounts of oil are required to kill either trees or insects, though it is agreed that the mineral-oil constituent left behind after spraying is the toxic agent. This paper gives the results of experiments conducted to develop a satisfactory method for the estimation of oil deposited upon foliage by spray emulsions.

## PRELIMINARY TESTS

English,<sup>2</sup> working with the leaves of Satsuma orange trees (*Citrus nobilis unshiu*), recently reported a method which is stated to be satisfactory for the determination of the oil retained upon plant surfaces. In this method, however, the natural oils and waxes that were extracted from the leaves along with the mineral oil that had been applied amounted to about 30 per cent of the total, so that subtraction of the volume of these natural substances was necessary in order to ascertain the amount of mineral oil deposited during spraying.

The first tests in the investigation reported here were made to determine the practicability of arriving at the quantity of oil retained by camphor leaves by finding the difference in the weights of individual leaves before and after spraying. Five leaves of camphor (*Cinnamomum camphora*) were detached from a garden tree at about 9 a. m. on June 13 and weighed at intervals to determine the losses due to evaporation. Table 1 gives the results.

The preliminary tests (Table 1) showed that the leaves lost from 14 to 26 per cent of their weight, owing to evaporation of moisture, in about eight hours. The conclusion was drawn that for this investigation the method of difference in weight of leaves could not be used, since the losses were of the same order of magnitude as the actual amounts of oil which might adhere to the surfaces.

<sup>1</sup> Received for publication Apr. 6, 1932; issued February, 1933.

<sup>2</sup> ENGLISH, L. L. METHOD FOR DETERMINING THE QUANTITY OF OIL RETAINED BY CITRUS FOLIAGE AFTER SPRAYING. Jour. Agr. Research 41: 131-133. 1930.

TABLE 1.—*Loss in weight of camphor leaves by evaporation*

Leaf No.	Initial weight		Weight at end of 1 hour		Weight at end of 2 hours		Weight at end of 4 hours		Weight at end of 8 hours	
	Gram	Per cent	Gram	Per cent	Gram	Per cent	Gram	Per cent	Gram	Per cent
1.....	0.5034	100	0.4958	98.49	0.4859	96.52	0.4664	92.65	0.4206	83.55
2.....	.4090	100	.3934	96.19	.3775	92.30	.3536	86.45	.3023	73.91
3 <sup>a</sup> .....	.4450	100	.4329	97.28	.4185	94.04	.3955	88.88	.3419	76.83
4 <sup>a</sup> .....	.5519	100	.5691	97.80	.5515	94.75	.5218	89.67	.4663	80.13
5 <sup>a</sup> .....	.5078	100	.5018	98.82	.4938	97.24	.4739	93.72	.4358	85.82

<sup>a</sup> Broken end dipped in paraffin.

Analyses were then made to obtain information concerning the variation and the proportion of waxes or natural oils on camphor leaves. The determination of natural wax was made as follows: Samples, each consisting of 100 disks, were cut by a specially constructed die, similar to that described by Ginsburg,<sup>3</sup> from leaves gathered at random. The diameter of the disks was 30 mm. The disks were placed in 200 c c Erlenmeyer flasks, and 50 c c of ether was added. The flasks and contents were so rotated that the disks were washed thoroughly, but no liquid was lost. The first washing was filtered into a 125 c c Erlenmeyer flask. The extraction was repeated twice with two 25 c c portions of ether. After the last ether extraction the filter paper was washed repeatedly with 10 c c portions of ether to remove the wax left on it. The extractions and washings were evaporated to dryness under reduced pressure on a water bath, care being taken to prevent the liquid from reaching the cork stopper. The residue after evaporation was redissolved in 25 c c of ether and filtered again into a 50 c c flask which had been previously dried and weighed. In all cases in which a transfer of ether solution was made from one flask to another, the original container was washed out at least three times with pure ether, and the mouth was wiped with filter paper after each washing. The filter papers used for this purpose were placed in the original flask. This procedure, which prevented the loss of the extracted substances by evaporation in pouring, was used throughout the work. The solution in the 50 c c flask was evaporated to dryness under reduced pressure on a water bath, and the flask was then removed, dried with a cloth while warm, and reweighed. The waxy residue was calculated by the difference in the weight of the 50 c c flask when empty and when containing the residue.

The following figures show the variation in the amount of natural wax on camphor leaves as determined by this method:

Sample No.	Weight of wax	Sample No.	Weight of wax
	Gram		Gram
1.....	0.0413	6.....	0.0331
2.....	.0362	7.....	.0356
3.....	.0333	8.....	.0397
4.....	.0391	9.....	.0383
5.....	.0352	10.....	.0351

The maximum variation from the average was 12.6 per cent.

<sup>3</sup> GINSBURG, J. M. AN APPARATUS FOR OBTAINING MEASURED AREAS OF SPRAYED FOLIAGE FOR CHEMICAL ANALYSES. *Jour. Agr. Research* 36: 1007-1009, illus. 1923.

In view of the fact that oil would actually be present during the analyses which follow, it was thought advisable to determine also what reliance might be placed on the figures representing the natural wax when the leaves were extracted in the presence of mineral oil. Accordingly, weighed amounts of Marcol oil were added to the sample prior to extraction, and these known weights of oil were subtracted from the total ether extract to obtain the values representing the wax. The results are given in Table 2. A maximum variation of about 30 per cent from the mean was found. The residue of natural wax was solid at room temperature, and produced a solidified mass when mixed with even three times its weight of mineral oil. The presence of oil during the analysis tended to increase the differences in the values obtained for wax content of the samples.

TABLE 2.—Wax extracted from camphor leaves with ether in the presence of oil

Sample No.	Weight of residue	Weight of oil added	Weight of wax by difference	Sample No.	Weight of residue	Weight of oil added	Weight of wax by difference
	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>		<i>Gram</i>	<i>Gram</i>	<i>Gram</i>
11.....	0.0605	0.0164	0.0441	17.....	0.0672	0.0625	0.0347
12.....	.0744	.0348	.0396	18.....	.1101	.0817	.0284
13.....	.0723	.0334	.0389	19.....	.1313	.1028	.0285
14.....	.0675	.0291	.0384	20.....	.1465	.1194	.0272
15.....	.0830	.0463	.0367	21.....	.1573	.1327	.0246
16.....	.0892	.0521	.0371	22.....	.1753	.1443	.0310

The variation in amount of natural wax obtained when extraction was made with chloroform in place of ether solvent in the method described above may be seen from the following tabulation:

Sample No.	Weight of wax	Sample No.	Weight of wax
	<i>Gram</i>		<i>Gram</i>
23.....	0.0939	27.....	0.0976
24.....	.0955	28.....	.0896
25.....	.1188	29.....	.0910
26.....	.0916	30.....	.1151

These amounts are of the same order of magnitude as the amount of oil which might be actually retained by the leaves, and the variation in wax content from sample to sample was proportionally large.

On the basis of such results it was deemed necessary to introduce into the analytical procedure steps which would bring about the removal of wax and permit the quantitative recovery of the pure mineral oil.

#### DETERMINATION OF OIL RETAINED BY CAMPHOR LEAVES

The two additional steps introduced are contained in the following method: 100 disks of 30-mm diameter were cut from leaves picked at random to make a single sample. Weighed amounts of Marcol oil (about 100 mg) were added with the extraction ether to the different samples. Each sample was washed for 1 minute with 50 c c of ether, as in the preceding method for the determination of wax, and then washed twice with 25 c c portions of ether to remove the last traces

of oil. The ether extractions were poured into a 125 c c Erlenmeyer flask and evaporated to dryness without filtering. The residue was dissolved at room temperature in a mixture of ether and absolute alcohol (1:1). On standing, a clear solution resulted. This was cooled to  $-30^{\circ}$  C. for 15 minutes or until waxy flakes precipitated. The precipitated wax together with undissolved dirt, etc., was then filtered through a suction funnel surrounded with a 22 per cent sodium chloride constant-temperature mixture maintained at about  $-20^{\circ}$  by small pieces of solid carbon dioxide (fig. 1) into another clean 125 c c Erlenmeyer flask.<sup>4</sup> The precipitated wax caught on the cold filter was washed three times with 10 c c portions of alcohol-ether mixture previously cooled down to  $-30^{\circ}$ . The clear filtrate and washings were evaporated to about 10 c c and transferred by means of a funnel to a previously calibrated Babcock skim-milk bottle.

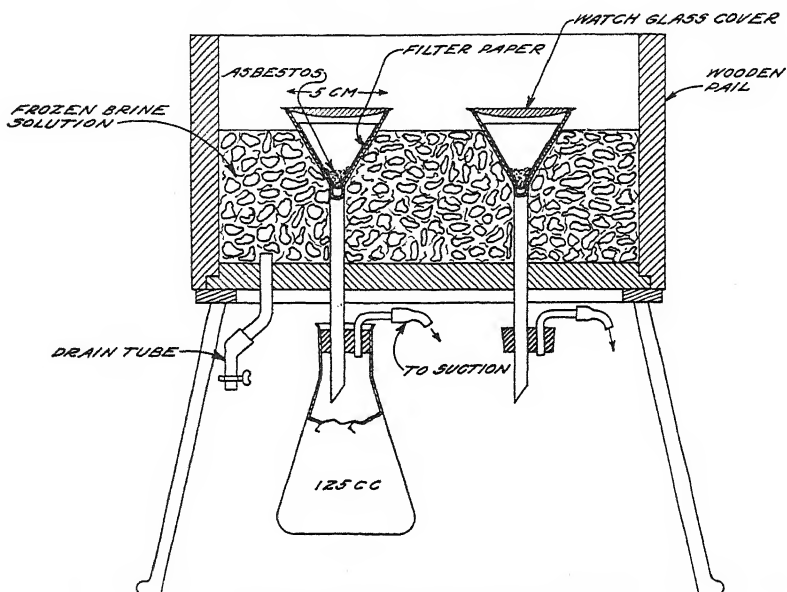


FIGURE 1.—Apparatus for cold filtration

The 125 c c Erlenmeyer flask was then washed three times with 5 c c portions of ether. The washings were poured into the Babcock bottle, care being taken to prevent loss by evaporation. By placing the forefinger over the mouth of the Babcock bottle and immersing the bottle in hot water the ether was made to evaporate through the capillary.

To the oily residue then in the Babcock bottle was added 6 $\frac{1}{2}$  c c (a few drops at first) of concentrated nitric acid (specific gravity 1.42), which initiated a violent reaction, partly destroying the unsaturated compounds and chlorophyll. The mixture, which consisted of acid, oil, and some partly destroyed organic matter, was raised to the boiling point by immersing the bottle in boiling water. After several agitations by hand, and after the oily layer appeared to be clear, the bottle was

<sup>4</sup> For the method of determination of paraffin, see the following publication: HOLDE, D. THE EXAMINATION OF HYDROCARBON OILS, AND OF SAPONIFIABLE FATS AND WAXES. Authorized transl. from German ed. 5, by E. Mueller. Eng. ed. 2, p. 108-109. New York. 1922.



filled to the neck with concentrated nitric acid and then centrifuged. The length of the column of oil was measured at room temperature by means of calipers equipped with a vernier permitting measurements to the nearest 0.1 mm. The skim-milk bottles of different sizes were calibrated by introducing mercury into the capillaries, measuring the length of the mercury column, recording the temperature of the air in the vicinity of the bottle, and calculating the volume per millimeter of length of each capillary. The specific gravity of the oil, which was determined to be 0.8447 at 15.5° C., was corrected 0.00061 per degree between 15.5° and room temperature, before converting the volume of oil measured into mass. The oil was clear and ran freely, but in some cases about 1 or 2 mm of solid matter not completely destroyed by the nitric acid came up into the capillaries. In such cases this wax or solid matter was measured as oil, since better results were so obtained. The oil used in all analyses was known to contain 97 per cent unsulphonatable matter. Blank tests showed, however, that the oil was not attacked by concentrated nitric acid under the conditions of these experiments, and correction for loss in oil due to the action of hot nitric acid was not necessary. Determinations of the oil in blank samples (containing no leaf waxes) agreed with the weights of oil taken, within 0.1 per cent, which was about the limit of error in observation, showing the calibrations also to be exact.

Table 3 shows the results obtained by this method in the determination of nine samples of oil that had been added to nine samples of camphor leaves. The error is comparatively large in the first four samples because the technic was not yet perfected.

TABLE 3.—*Recovery of oil previously added to samples of camphor leaves*

(100 disks per sample, 3 cm diameter)

## FIRST SET

Sample No.	Length of oil column	Volume per millimeter of capillary	Weight of oil added	Calculated weight of oil recovered	Error
	<i>Mm</i>	<i>Cc</i>	<i>Gram</i>	<i>Gram</i>	<i>Per cent</i>
31.....	76.1	0.001552	0.1004	0.0995	-0.89
32.....	89.0	.001477	.1121	.1105	-1.43
33.....	57.7	.001616	.0803	.0784	-2.36
34.....	77.8	.001552	.0998	.1017	+1.90

## SECOND SET

35.....	59.5	0.001616	0.0805	0.0806	+0.12
36.....	75.3	.001577	.1000	.0995	-.50
37.....	81.3	.001609	.1107	.1102	-.45
38.....	76.3	.001552	.1004	.1003	-.10
39.....	89.0	.001477	.1121	.1105	-1.43

## DETERMINATION OF OIL RETAINED BY PECAN LEAVES

That the method outlined above is also applicable to the determination of oil retained by pecan (*Hicoria pecan*) foliage following spraying is shown by the results in Table 4. No changes in the camphor-leaf method of determination were made except to heat the Babcock bottles, after nitric acid was added, to a temperature slightly higher than that used in the determination of oil on camphor leaves, in

order to destroy completely the acid-soluble wax. The bottles were heated and agitated directly over an electric heater until the acid began to boil.

TABLE 4.—*Recovery of oil previously added to samples of pecan leaves*  
(100 disks per sample, 3 cm diameter)

Sample No.	Length of oil column	Volume per millimeter of capillary	Weight of oil added	Calculated weight of oil recovered	Error
	<i>Mm</i>	<i>Cc</i>	<i>Gram</i>	<i>Gram</i>	<i>Per cent</i>
1.....	77.5	0.001616	0.1057	0.1051	-0.57
2.....	66.2	.001609	.0891	.0894	+ .34
3.....	66.0	.001577	.0871	.0875	+ .46
4.....	84.5	.001477	.1052	.1048	- .38
5.....	70.5	.001464	.0876	.0868	- .91
6.....	63.5	.001616	.0875	.0868	- .80
7.....	70.5	.001609	.0963	.0954	- .93
8.....	56.6	.001477	.0998	.0702	+ .57
9.....	78.1	.001616	.1056	.1060	+ .38
10.....	57.0	.001577	.0755	.0756	+ .13

#### DETERMINATION OF OIL RETAINED BY SATSUMA ORANGE LEAVES

As the application of the preceding methods to the determination of oil retained on Satsuma orange leaves was not successful, modifications were necessary. In preliminary tests, about 15 per cent of the oil added to the samples could not be recovered, as it was retained upon the disks after extraction and also upon the cold filter with the precipitated waxes. The first difficulty was avoided by extracting the leaf disks four times with 50 c c portions of ether instead of with one 50 c c portion and then with two 25 c c portions. As a matter of fact, 25 c c would not completely cover the disks. Though it was feasible to pass the cold extractions from the pecan leaves and the extractions from the camphor leaves, in a 1:1 alcohol-ether solvent, directly through the cold filter without previously filtering out dirt, scales, and other residue insoluble in ether, the same impurities, in the case of orange leaves, had to be filtered out immediately after extraction. It was found that such foreign matter promoted the occlusion of mineral oil in the wax precipitated later. With these precautions the error was reduced to about 7 per cent, but the results were far from satisfactory.

In order further to determine the optimum conditions under which a complete separation of natural wax and oil could be attained with Satsuma orange leaves, several additional trials were made in which the composition of the solvent used in freezing out the waxes and the temperature of the freezing bath were varied. Table 5 shows the results from some of these tests.

The results of the trial tests showed that a ratio of 20 c c alcohol to 10 c c ether gave the most effective solvent in which to precipitate the wax regardless of the temperature of the freezing mixture, whereas the optimum temperature of the freezing mixture was between -6° and -10° C. Above this temperature wax insoluble in nitric acid passed through the filter, and below it oil was retained on the filter with wax. Constant-temperature freezing mixtures were obtained by mixing different proportions of brine with water, and constant temperature was maintained by the use of solid carbon dioxide.

TABLE 5.—*Recovery of oil previously added to samples of Satsuma orange leaves, using different alcohol-ether mixtures at different temperatures*

(75 disks per sample, 3 cm diameter)

## FIRST SET

Sample No.	Volume of alcohol	Volume of ether	Temperature of precipitation	Weight of oil added	Calculated weight of oil recovered	Error
	C c	C c	° C.	Gram	Gram	Per cent
1.....	5	15	-20	0.0835	0.0688	-17.6
2.....	10	10	-20	.0655	.0554	-15.4
3.....	15	10	-20	.0680	.0640	-5.9
4.....	20	10	-12	.0701	.0654	-6.7
5.....	20	10	-6	.0665	.0660	-.75

## SECOND SET

6.....	10	15	-20	.0698	.0640	-8.31
7.....	15	10	-20	.0647	.0627	-3.09
8.....	20	5	-20	.0679	.0634	-6.63

## THIRD SET

9.....	20	10	-8	.0630	.0629	-.15
10.....	20	10	-8	.0641	.0635	-.94
11.....	20	10	-8	.0652	.0643	-1.38

The order of magnitude of error involved in the determination of oil on Satsuma orange leaves under the optimum conditions may be seen from Table 6. Because it was impossible to recover all the oil, a correction of 1.5 per cent has been added. In general this correction gave better values. It would therefore be necessary in the analysis of unknown samples to add such a correction to the results. Even then a maximum error of 2.5 per cent is possible.

TABLE 6.—*Recovery of oil previously added to samples of Satsuma orange leaves under optimum conditions*

(75 disks per sample, 3 cm diameter)

Sample No.	Length of oil column	Volume per millimeter of capillary	Weight of oil added	Calculated weight of oil recovered	Error
	Mm	C c	Gram	Gram	Per cent
12.....	44.0	0.001616	0.0617	0.0606	-1.78
13.....	47.0	.001609	.0630	.0645	+2.38
14.....	52.0	.001616	.0706	.0716	+1.42
15.....	53.0	.001477	.0657	.0667	+1.52
16.....	50.4	.001616	.0702	.0693	-1.28
17.....	52.0	.001578	.0686	.0700	+2.04
18.....	47.7	.001609	.0666	.0655	-1.65
19.....	54.3	.001477	.0700	.0683	-2.43
20.....	50.5	.001463	.0641	.0630	-1.72
21.....	47.0	.001809	.0657	.0645	-1.83

## DISCUSSION

Before analyses of the type referred to are made, it is believed desirable to run a number of check samples in which the quantity of oil is known, in order to secure the correct working conditions and

technic. Naturally the methods, as given, are not applicable to the determination of unrefined oils (containing a relatively small proportion of unsulphonatable compounds) unless it can be shown that nitric acid either destroys a constant known percentage of the oil under consideration or does not attack the oil at all, as was the case in the present investigation. Although most oils used in spraying do contain appreciable amounts of unsaturated compounds, it is possible to select such refined oils in the study of the physical characteristics of emulsions as will not necessitate taking into account the action of nitric acid on the oil.

To illustrate one of the countless uses to which information concerning the retention of oil by trees may be applied, the following example is cited. It shows how the amount of oil deposited on camphor trees by sodium oleate emulsions containing different amounts of emulsifier changes with the concentration of the emulsifier. Six groups of nursery trees about 6 feet high, three trees to the group, were sprayed on August 10 with six different emulsions, trees of each group being sprayed with the same emulsion. In all six emulsions the concentration of Marcol mineral oil was constant (1.6 per cent), but the concentration of sodium oleate was different. Distilled water was used in the preparation and dilution. The soap was prepared directly from U. S. P. oleic acid and sodium hydroxide. A recently developed method<sup>5</sup> for spontaneously dispersing oil in water which obviates prolonged agitation was employed. After spraying, a sample of leaves was collected from each tree, three samples for each emulsion. The data in Table 7 are self-explanatory in respect to the number of leaf disks which composed the samples, the amount of soap contained in each emulsion, and the oil recovered.

TABLE 7.—Oil retained on camphor leaves after spraying with emulsions of different concentrations

Group	Emulsion No.	Normality of soap $\times 10^4$	Leaf disks per sample	Weight of oil found	Grams oil per $\text{Cm}^2$ <sup>a</sup> of leaf <sup>b</sup> $\times 10^5$
			Number	Gram	
1.-----	1	29.8	100	0.0708	10.01
	2			.0713	10.08
	3			.0720	10.18
2.-----	1	24.8	100	.1090	14.56
	2			.1010	14.28
	3			.1075	15.20
3.-----	1	17.38	75	.1063	20.05
	2			.1078	20.33
	3			.1030	19.42
4.-----	1	12.40	75	.1143	21.55
	2			.1085	20.46
	3			.1228	23.16
5.-----	1	7.44	50	.0854	24.16
	2			.0843	23.85
	3			.0826	23.37
6.-----	1	3.72	25	.0428	24.21
	2			.0432	24.43
	3			.0418	23.65

<sup>a</sup>  $\text{Cm}^2$  is the abbreviation for square centimeter recently adopted by the Style Manual for U. S. Government Printing Office.

<sup>b</sup>  $1 \text{ cm}^2$  of leaf =  $2 \text{ cm}^2$  actual leaf surface, therefore if concentration of oil on leaf surface is considered, the results in this column should be divided by 2.

<sup>5</sup> An account of this method has not yet been published. These emulsions are comparable in physical properties to emulsions prepared by the usual methods and possess several advantages over them.

From the data in Table 7 a curve has been plotted showing the variation in oil deposited with changing emulsifier concentration. (Fig. 2.)

#### SUMMARY

The quantity of naturally occurring waxes extracted from camphor leaves by ether and by chloroform has been shown to vary in different samples with equal areas of leaf surface.

A method is given for the determination of the quantity of paraffin mineral oil retained by camphor leaves after spraying. The oil is

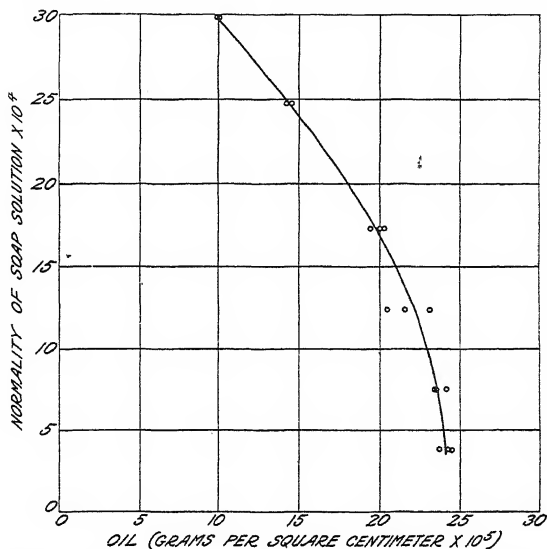


FIGURE 2.—Relation between emulsifier concentration and amount of oil deposited

recovered from the leaves in its original form, and the amount is measured volumetrically in calibrated Babcock skim-milk bottles.

By introducing certain variations the method is applicable to pecan leaves and Satsuma orange leaves. The maximum error in determining oil on pecan leaves was less than 1 per cent; on camphor leaves and Satsuma orange leaves it was less than 2.5 per cent.

An example is given illustrating the use of the method in estimating oil retained by camphor-tree foliage that had been sprayed with emulsions.



# DIFFERENCES IN THE AMINO ACID CONTENT OF THE CHIEF PROTEIN (GLYCININ) FROM SEEDS OF SEVERAL VARIETIES OF SOYBEAN<sup>1</sup>

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## INTRODUCTION

The enormously increased production of soybeans (*Soja max*) in the United States during recent years has emphasized their importance as an economic crop. A large proportion of the seeds produced are used as a source of oil, and the press cake which is obtained as a by-product is ground and sold as soybean meal.

Soybean meal is recognized as one of the best protein concentrates for stock feeding. It has been amply demonstrated, both by experimental studies in the laboratory and by the practical feeding of farm animals, that the proteins of the soybean have a high nutritive value.

Proteins differ widely in their nutritive value, depending chiefly on the quantities of certain amino acids which they yield on digestion. The chief proteins of some of our most important foodstuffs are deficient in one or more of the so-called nutritionally essential amino acids. Classic examples are zein from corn, deficient in lysine and tryptophane, and gliadin from wheat, deficient in lysine. Phaseolin, the chief protein of the navy bean, is deficient in cystine. It has been shown in previous publications from this division that the proteins of several other legume seeds, including the Lima bean, mung bean, velvetbean, adzuki bean, lentil, and cowpea, are likewise deficient in cystine. The soybean proteins, on the other hand, have been generally considered to contain adequate amounts of all the known essential amino acids. Mitchell and Smuts (5),<sup>2</sup> however, have recently reported that soybeans are rather low in cystine content.

The unusually wide range of differences in the characteristics of several soybean varieties has raised the question whether there may be a difference in the nutritional value of the protein of different varieties. In the case of the proteins of wheat, it is known, for instance, that gliadin and glutenin obtained from different varieties and classes of wheat have the same chemical composition. It is equally well known that different classes of wheat, and wheat grown in different environments, vary not only in their total protein content but also in their ratio of gliadin to glutenin. These two proteins, however, irrespective of the quantity or the ratio of each in the wheat, have always been found to have practically the same composition. Because of the lower nutritional value of gliadin, a variety of wheat having a high ratio of gliadin to glutenin would have a lower protein value than a variety which had a higher proportion of glutenin.

<sup>1</sup> Received for publication Apr. 6, 1932; issued February, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 55.

Similarly, soybeans, like most other seeds which have been investigated, contain several proteins which differ in chemical composition. Any difference found in the protein nutritional value of one variety over that of another would, therefore, be explainable on the assumption that these proteins occur in different proportions in the different soybean varieties. Information relative to possible differences in the nutritive value of various varieties from this standpoint would be of importance in the selection of varieties grown for the production of seed intended for use as a food or feed. The investigation described in this article was undertaken with the object of obtaining data on this question.

Protein concentrates as generally used for feed are not fed as the sole source of protein in the ration, but as supplements to other feeds having a lower protein content. It is important, therefore, that the proteins of the concentrates contain not merely enough of the essential amino acids to supply the nutritional requirements of the animal, but that they have even more, so that the excess may be available to compensate for possible deficiencies in the other protein-containing ingredients of the diet.

Osborne and Campbell (?) found that the chief protein of soybeans consists of a globulin which they named glycinin. This protein amounted to 16.6 per cent of the meal. Evidence was also found of the presence of a second globulin, which was more soluble than glycinin and which could be obtained from the solution from which glycinin had separated on dialysis. In addition to these two globulins there was isolated a legumelin, amounting to about 1.5 per cent of the seed, and a small quantity of protease.

Glycinin, representing the globulin fraction obtained by dialyzing a saline extract of soybean meal, has been generally considered an individual protein. It constitutes the greater part of the total protein content of the soybean. Unpublished data recently obtained in this division indicate that glycinin thus prepared is not a single protein, but that it consists of at least two globulins. Furthermore, in a comparative study of the isoelectric points of different proteins, Csonka, Murphy, and Jones (1) found that glycinin was one of the few globulins studied which showed a deviation from the narrow range in isoelectric points characteristic of globulins as a class. This further supports the conclusion that glycinin is not an individual protein.

In the work here reported it was decided to limit the study to analyses of glycinin preparations similarly obtained from the different varieties. The ideal way would have been not to select any one protein or group of proteins, but to apply the analyses to the total protein content of the soybean. Amino acid determinations, however, can not be made even with approximate accuracy on the whole seed but must be made on the isolated proteins, therefore, the protein fraction selected for study was that which represents the greater part of the total protein of the soybean and which can most readily be isolated.

Of the three amino acids determined in the several glycinin preparations, cystine and tryptophane are known to be nutritionally essential. The percentages of these amino acids in the glycinin preparations can therefore serve as criteria of importance in a consideration of the relative nutritive value of the proteins of the different soybean varieties.



## MATERIAL AND METHODS

The soybeans used in this investigation were supplied by the division of forage crops and diseases of the Bureau of Plant Industry, and the varieties were selected on the merit of their widespread popularity among the soybean growers of the United States. The samples were obtained from localities in which the environmental conditions were known to be suited for the optimum growth and development of the varieties studied. The characteristics of these varieties are described in a recent publication by Morse (6).

The soybeans were ground to a meal, and the fat was removed by extraction with ether. The fat-free material was then extracted with 10 per cent sodium chloride solution. The saline extract was filtered by suction through a mat of filter-paper pulp, and the clear filtrate was dialyzed for 10 to 14 days. The glycinin which separated on dialysis was removed by centrifugation and washed several times with distilled water. It was finally dehydrated in the usual way with ethyl alcohol followed by ether.

Cystine, tyrosine, and tryptophane were determined in the different glycinin preparations by colorimetric methods. The method of Folin and Ciocalteu (2) was used for the tyrosine determination, that of Sullivan (8) for cystine, and May and Rose's method (4), with slight modification, for tryptophane. The maximum color development in the tryptophane determinations occurred after the casein standard and the glycinin sample had stood for five days at 38° C. The results of the analyses are given in Table 1.

TABLE 1.—*Tryptophane, cystine, tyrosine, and nitrogen content of glycinin prepared from different varieties of soybean seed expressed in percentages of moisture and ash-free protein*

Variety	Color of seed	Tryp-to- phane	Cys- tine	Tyro- sine	Ni- tro- gen	Variety	Color of seed	Tryp-to- phane	Cys- tine	Tyro- sine	Ni- tro- gen
Peking.....	Black.....	2.03	0.81	3.94	17.19	Mammoth	Straw yel-	1.89	0.92	4.44	17.30
Illini.....	Straw yel-	2.84	.74	4.55	17.74	Yellow,	low.....				
A. K.....	do.....	2.26	1.17	4.38	17.41	Haberlandt	do.....	2.24	.98	4.02	17.13
Manchu.....	do.....	2.36	1.45	4.36	17.28	Dunfield.....	do.....	2.20	.98	4.26	17.37
Virginia.....	Brown.....	2.28	.95	4.49	17.43	Dixie.....	do.....	2.16	.93	4.22	16.60
						Chiquita.....	do.....	1.94	1.46	4.31	17.16

## RESULTS OF ANALYSES

Although the differences found in the percentages of tryptophane and cystine in the glycinin preparations obtained from the soybean varieties studied are not great on the whole, some differences were found which are greater than can well be attributed to experimental error involved in the analytical procedures employed. Tryptophane, cystine, and tyrosine values determined on the same protein by the colorimetric methods used, particularly when the determinations are carried out by the same person under as nearly as possible identical conditions, may be expected to be within a limit of error of 2 to 3 per cent. The tryptophane values found range from 1.89 per cent for the Mammoth Yellow variety to 2.84 per cent for the Illini variety, a difference of nearly 1 per cent. The difference in tryptophane content between the Illini variety and the other varieties studied is great enough to be of nutritional significance.

The cystine values, on the other hand, range from 0.74 per cent for the Illini variety to 1.46 per cent for the Chiquita variety, a difference of 0.72 per cent. The Chiquita and Manchu varieties contain significantly higher percentages of cystine than the other varieties. It is of interest to note that of the varieties studied, the Illini variety, which contained the highest percentage of tryptophane, contained the lowest percentage of cystine, and that the Chiquita variety contained the highest percentage of cystine, but was among the lowest in tryptophane. The Peking and Dixie varieties were low in both cystine and tryptophane.

The cystine and tryptophane values found for the glycinin fraction of the soybeans studied are of significance from the standpoint of practical feeding. The Illini variety, because of its higher tryptophane content, should prove to be more valuable than the other varieties as a supplement to feeds, such as corn and oats, which are low in tryptophane. Feedstuffs which are deficient in cystine, such as cowpeas, lentils, and peas, should benefit more by supplementation with Chiquita and Manchu soybeans than with the Peking and Illini varieties, which contain only about one-half as much cystine.

In considering the application of the values found for these two nutritionally essential amino acids to problems related to the feeding of soybeans, it must be remembered that the determinations were made on glycinin, and that, therefore, the values may not be quite typical of the total protein content of the seed. There are proteins other than glycinin present in soybeans concerning the composition of which we have little knowledge. It is possible that the tryptophane and cystine content of these proteins may be quite different from that of glycinin. In view of the fact, however, that these proteins are present in relatively small quantities, it is not probable that they affect much, one way or the other, the amino acid value of the whole soybean seed.

The glycinin preparations obtained from the different varieties agree closely in their nitrogen content with the exception of that of the Dixie and Illini varieties, the former containing the highest and the latter the lowest percentage of nitrogen. There is no apparent correlation between either the nitrogen and amino acid content of the different glycinin preparations or between the quantities of the three amino acids. As pointed out in a preceding paragraph, glycinin is probably not an individual protein but represents a mixture of two or more globulins. These globulins undoubtedly differ in their chemical composition. The variations found in the amino acid percentages obtained from the several varieties studied are then easily explainable on the ground of variations in the relative proportions in which the different globulins comprising the glycinin fractions are present in the different varieties of soybeans.

## DISCUSSION

In view of the fact that the different varieties of soybeans studied were not grown in the same localities, it is recognized that the differences found in the amino acid content of the glycinin preparations obtained from them are not necessarily characteristic of the varieties but may be due to the effect of external environmental conditions. Some varieties grow best in a warm climate, others do better in a cool climate.

The soybeans studied were grown in localities best suited for the growth and culture of the particular varieties. A large part of the total production of these varieties in the United States is located in those sections from which the samples analyzed were obtained. It is therefore believed that the results here presented are representative of these varieties as they are found on the market, and that, therefore, they have a greater value to the producer and consumer than if the different varieties had been grown in one place under identical environmental conditions.

The oil extracted from the soybeans used in this investigation has been studied by the Oil, Fat, and Wax Laboratory of this bureau, and the results are presented in another paper (3).

#### SUMMARY

The results of colorimetric determinations of cystine, tryptophane, and tyrosine in glycinin preparations obtained from 10 different varieties of soybeans are reported.

The percentages of cystine, tryptophane, and tyrosine found for most of the varieties studied are in fairly close agreement. Differences between some varieties were found, however, which are greater than can well be attributed to experimental error involved in the analytical procedures employed. Tryptophane values ranged from 1.89 per cent for the Mammoth Yellow variety to 2.84 per cent for the Illini variety. The cystine values ranged from 0.74 per cent for the Illini variety to 1.46 per cent for the Chiquita variety.

The divergencies found in the amino acid content of the different glycinin preparations are explainable on the ground of variations in the relative proportions in which the different globulins comprising the glycinin fractions are present in the different varieties of soybeans.

The significance of the variations is discussed from the standpoint of nutritional value.

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# OIL CONTENT OF NINE VARIETIES OF SOYBEAN AND THE CHARACTERISTICS OF THE EXTRACTED OILS<sup>1</sup>

By G. S. JAMIESON, *Senior Chemist*, W. F. BAUGHMAN, *Associate Chemist*, and R. S. MCKINNEY, *Assistant Chemist, Oil, Fat, and Wax Laboratory, Bureau of Chemistry and Soils, United States Department of Agriculture*

It was considered of interest to determine the oil content of several varieties of soybean (*Soja max*) used in the protein investigation reported in another paper,<sup>2</sup> and to ascertain the more important chemical and physical characteristics of the oils extracted.

The oil content of the beans was determined in the usual manner by extraction with petroleum ether, but the oils upon which the characteristics were determined were obtained by ether extraction, because this solvent was preferable to the other for the preparation of the ground beans in the protein investigation. In the case of soybeans, however, there is little difference in the quantities of nonoil constituents extracted along with the oil by the use of either solvent. The ether was removed as completely as possible by distillation, and the remainder was removed by heating the oils in an oven in an atmosphere of carbon dioxide, at a temperature of about 115° C. until a constant weight was obtained. The results are given in Table 1.

TABLE 1.—*Chemical and physical properties of soybeans and their oils*<sup>a</sup>

## CHEMICAL AND PHYSICAL CHARACTERISTICS OF OILS

Item	Dun-field	Man-chu	Haber-landt	Vir-ginia	Chi-quita	Mam-moth Yellow	Peking	Illini	A. K.
Specific gravity...25°/25° C...	0.9216	0.9218	0.9196	0.9198	0.9245	0.9224	-----	-----	-----
Refractive index at 20° C....	1.4748	1.4749	1.4745	1.4743	1.4763	1.4748	1.4763	1.4749	1.4756
Acid value.....	-----	-----	-----	-----	-----	-----	1.4	1.5	1.6
Saponification value.....	191.2	191.6	191.6	191.5	191.1	191.7	190.0	189.9	190.9
Unsaponifiable matter.....per cent..	.73	.88	.80	1.08	.85	.85	1.10	.86	.89
Iodine number (Hanus).....	131.4	131.0	131.6	127.8	140.7	129.4	141.4	131.3	132.6
Thiocyanogen-iodine number.....	84.1	82.2	82.2	81.4	87.0	83.4	-----	-----	-----
Saturated acids.....per cent..	12.6	11.9	12.5	12.5	11.7	11.7	-----	-----	-----
Unsaturated acids.....per cent..	80.4	80.9	80.2	80.1	81.0	80.8	-----	-----	-----
Iodine number of unsaturated acids (calculated).....	162.6	161.5	162.1	157.8	172.4	159.1	-----	-----	-----

## MOISTURE CONTENT AND OIL CONTENT OF BEANS

Moisture.....per cent..	5.87	5.62	5.40	6.46	7.24	6.93	5.61	7.30	6.00
Oil.....per cent..	18.86	18.12	18.37	19.13	16.79	15.61	17.98	19.28	21.85

<sup>a</sup> Analyses of beans and oil were made by R. S. McKinney and W. F. Baughman, oil, fat, and wax laboratory. The oils were extracted by ether so that the proteins could be investigated.

<sup>1</sup> Received for publication Apr. 6, 1932; issued February, 1933.

<sup>2</sup> OSONKA, F. A., and JONES, D. B. DIFFERENCES IN THE AMINO ACID CONTENT OF THE CHIEF PROTEIN (GLYCININ) FROM SEEDS OF SEVERAL VARIETIES OF SOYBEAN. *Jour. Agr. Research* 46: 15-55, 1933.

It will be observed from the table that the most striking differences found in the oils studied is in the iodine numbers and the thio-cyanogen values. These differences are due to the variation in the proportions of oleic, linoleic, and linolenic acids in these oils. The oil from the seed of the Virginia variety has the lowest iodine number and the lowest refractive index, whereas the oils from the seed of the Chiquita and Peking varieties have the highest values for these characteristics.

Although the oil content of the seed from the different varieties shows a considerable range, the similarity in the proportions of saturated and unsaturated acids is noteworthy.

# RELATION OF WEATHER TO THE PREVALENCE OF WHEAT STEM RUST IN NEBRASKA<sup>1</sup>

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## INTRODUCTION

For the 10-year period 1921-1930 detailed observations were made on the appearance, subsequent development, and the prevalence of wheat stem rust (*Puccinia graminis tritici* (Pers.) Erikss. and Henn.) in Nebraska.<sup>2</sup>

This period is a rather short one for an analysis of the relation of weather to stem-rust development, in view of the conclusions reached by Lambert,<sup>3</sup> based on a study of stem rust during a 22-year period. He says that no evidence was obtained of any specific meteorological condition or set of conditions that always accompanies epidemics. "With so many interrelated factors involved, each epidemic appears to be a law unto itself. More precise information is needed, but the observer can not be cautioned too strongly against mistaking coincidences for causal relations." It can be assumed that certain factors must be contributing agents to the development of stem rust, since epidemics are the result of a progressive series or a combination of conditions favoring the rapid spread and development of stem rust.

The more important factors which may influence the development of stem rust of wheat in Nebraska are as follows: (1) The amount and regional distribution of initial inoculum reaching the wheat fields together with the time of its appearance; (2) the temperature and moisture favorable for the germination of the spores and subsequent infection; and (3) the amount and distribution of primary urediospores and the environmental factors suitable for the optimum development of the host and parasite, together with optimum conditions favorable for reinfection of the secondary and succeeding generations of urediospores. It is the purpose of this paper to discuss the influence of some of the above-mentioned factors on the prevalence of wheat stem rust and to point out the conditions under which rust epidemics may occur in Nebraska.

## RELATION OF SEASONAL VARIATION IN THE TIME AND LENGTH OF THE FRUITING PERIOD TO STEM-RUST EPIDEMICS

Before discussing the environmental factors influencing the development of stem rust, it might be well to point out the conditions that affect the time and length of the fruiting period of winter wheat. Observations over a 10-year period have shown that primary uredia

<sup>1</sup> Received for publication Apr. 26, 1932; issued February, 1933. Paper No. 120 of the Journal series of the Nebraska Agricultural Experiment Station.

<sup>2</sup> PELTIER, G. L. PHYSIOLOGIC FORMS OF WHEAT STEM RUST IN KANSAS AND NEBRASKA. Unpublished manuscript. — and THIEL, A. F. STEM RUST IN NEBRASKA. PART I. GENERAL SURVEY OF SOURCES. PART II. IDENTIFICATION OF THE PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS FROM VARIOUS SOURCES. Nebr. Agr. Expt. St. Research Bul. 42, 40 p., illus. 1927.

<sup>3</sup> LAMBERT, E. B. THE RELATION OF WEATHER TO THE DEVELOPMENT OF STEM RUST IN THE MISSISSIPPI VALLEY. Phytopathology 19: 1-71, illus. 1929.

have never been found in the spring on winter wheat in Nebraska prior to the late-boot or early-heading stage. Thus, with the exception of the environmental factors necessary for initial infection, the variations in the time and length of the fruiting period may be one of the major factors in determining the development and prevalence of stem rust in Nebraska.

TABLE 1.—Seasonal variation in the time and length of the fruiting period of Turkey Red winter wheat<sup>a</sup> during 28 years at Lincoln, Nebr

Year	Date of heading	Date of ripening	Length of fruiting period	Yield per acre
			Days	Bushels
1903.....	June 2.....	July 11.....	39	32
1904.....	June 4.....	July 13.....	39	18
1905.....	May 29.....	July 2.....	34	21
1906.....	May 28.....	July 3.....	36	62
1907.....	June 7.....	July 8.....	31	52
1908.....	May 29.....	June 29.....	31	38
1909.....	June 17.....	July 13.....	26	27
1910.....	May 31.....	July 2.....	32	48
1911.....	May 30.....	June 23.....	24	9
1912.....	May 31.....	July 5.....	35	9
1913.....	May 30.....	June 28.....	29	50
1914.....	June 1.....	June 25.....	24	34
1915.....	.....do.....	July 8.....	37	30
1916.....	June 2.....	July 7.....	35	38
1917.....	June 17.....	July 17.....	30	32
1918.....	May 26.....	June 27.....	32	19
1919.....	June 3.....	July 3.....	30	26
1920.....	June 9.....	July 7.....	28	37
1921.....	May 26.....	June 20.....	25	41
1922.....	May 28.....	June 24.....	27	31
1923.....	June 6.....	July 6.....	30	24
1924.....	June 3.....	.....do.....	33	33
1925.....	May 29.....	June 23.....	25	15
1926.....	May 28.....	June 24.....	27	14
1927.....	June 7.....	July 6.....	29	37
1928.....	May 29.....	July 3.....	35	24
1929.....	June 8.....	.....do.....	30	34
1930.....	May 27.....	June 30.....	34	43
Average.....	June 2.....	July 3.....	31	33

<sup>a</sup> Crop considered in head or ripe when approximately 80 per cent of the culms had reached the specified condition. These are composite results for ordinary Turkey Red wheat grown on the Nebraska Experiment Station farm, at Lincoln. The 1917 winter-wheat yield is the average for 3 fields of wheat that did not winterkill. The wheat in a fourth field, representing about one-third of the total acreage, winterkilled, and the field was replanted to corn. These data were supplied by Dr. T. A. Kiesselbach, agronomist, Nebraska Agricultural Experiment Station. The data prior to 1924 have been reported by him in the following publication: KIESELBACH, T. A. WINTER WHEAT INVESTIGATIONS. Nebr. Agr. Expt. Sta. Research Bul. 31, 149 p., illus.

Table 1 shows the time of heading and ripening and the length of the intervening period for Turkey Red wheat for 28 years (1903–1930) at Lincoln, Nebr. There is a range of 22 days in the time of heading (May 26, in 1921, and June 17, in 1917). The average date of heading for the entire period is June 2. Likewise, there is a range of 27 days in the time of ripening (June 20, in 1921, and July 17, in 1917). The average date of ripening for the 28 years is July 3. The length of the fruiting period varies from 24 to 39 days, with a range of 15 days. The average length of the fruiting period is 31 days. As is to be expected, the yields obtained ranged from 9 bushels in 1912 to 62 bushels in 1906, with an average of 33 bushels. With such wide variations in the time of heading and ripening and the length of the fruiting period, it seems likely that the prevalence of stem rust from year to year would also vary greatly, because the environmental factors responsible for the seasonal variations in the development of



the wheat plant from heading to maturity would also influence the development and prevalence of stem rust.

To ascertain the influence of weather upon the length of the fruiting period, the mean temperature, amount of precipitation, and number of rainy days for the actual period for each of the 28 years are given in Table 2. It is apparent that with few exceptions there is a relation between mean temperatures and length of the fruiting period, the shorter periods having higher mean temperatures than the longer ones. Thus the average mean temperature for the period of 24 to 27 days is near 75° F., whereas for the period of 36 to 39 days it is about 69°, about 6° less. The direct influence of precipitation can not be determined because of its variability from year to year. The same may be said for the number of rainy days. There is a tendency, however, toward the occurrence of high rainfall and numerous rainy days along with the lower mean temperatures and extended periods of fruiting. There are, however, two exceptions to this statement—an extremely high rainfall occurred with a high mean temperature in 1914 and a low rainfall with a low mean temperature in 1903.

TABLE 2.—*Influence of weather on the length of the fruiting period of Turkey Red winter wheat at Lincoln, Nebr.*

LENGTH OF FRUITING PERIOD 24 TO 27 DAYS

Year	Actual length of fruiting period	Mean temperature	Precipitation	Rainy days
	Days	° F.	Inches	Number
1909.....	26	76.5	4.90	10
1911.....	24	77.4	.56	6
1914.....	24	76.0	10.06	14
1921.....	25	75.0	4.40	9
1922.....	27	74.8	1.27	8
1925.....	25	75.6	6.15	12
1926.....	27	70.1	2.65	9
Average.....	25	75.1	4.28	10

LENGTH OF FRUITING PERIOD 28 TO 31 DAYS

1907.....	31	73.8	5.38	9
1908.....	31	68.4	11.33	20
1913.....	29	73.7	2.02	9
1917.....	30	74.7	3.50	6
1919.....	30	75.7	3.74	11
1920.....	28	75.2	2.10	10
1923.....	30	72.5	.79	8
1927.....	29	72.4	3.08	7
1929.....	30	72.4	.93	7
Average.....	30	73.2	3.65	10

LENGTH OF FRUITING PERIOD 32 TO 35 DAYS

1905.....	34	70.6	4.75	12
1910.....	32	72.3	2.57	7
1912.....	35	73.1	3.15	12
1916.....	35	70.5	3.14	11
1918.....	32	76.8	2.54	11
1924.....	33	69.1	4.22	16
1928.....	35	68.2	3.97	17
1930.....	34	69.7	3.39	9
Average.....	34	71.3	3.47	12

TABLE 2.—*Influence of weather on the length of the fruiting period of Turkey Red winter wheat at Lincoln, Nebr.—Continued*

## LENGTH OF FRUITING PERIOD 36 TO 39 DAYS

Year	Actual length of fruiting period	Mean temperature	Precipitation	Rainy days
	Days	° F.	Inches	Number
1903.....	39	70.5	2.32	12
1904.....	39	69.2	6.85	21
1906.....	36	69.9	7.50	12
1915.....	37	67.4	5.12	18
Average.....	38	69.3	5.45	16

While it is true that the mean temperatures during the fruiting period determine to some extent the length of this period, it is of interest to know what the weather conditions were prior to and during the interval from heading to maturity. Therefore, the weather data for the months of May, June, and July for each of the 28 years, and the departures from normal are given in Table 3, grouped as in Table 2. The data show that where the length of the fruiting period was from 24 to 27 days, the mean temperatures, with few exceptions, were above normal in all three months, whereas the precipitation and number of rainy days were in general below normal. The year 1914 was exceptional, since an excess of rain occurred during each of the three months.

TABLE 3.—*Departures from normal of mean temperatures, amount of precipitation, and number of rainy days for the months of May, June, and July for a 28-year period at Lincoln, Nebr.<sup>a</sup>*

## LENGTH OF FRUITING PERIOD 24 TO 27 DAYS

Year	May			June			July			Total departure		
	Temperature	Precipitation	Rainy days	Temperature	Precipitation	Rainy days	Temperature	Precipitation	Rainy days	Temperature	Precipitation	Rainy days
	°F.	Inches	Number	°F.	Inches	Number	°F.	Inches	Number	°F.	Inches	Number
1909.....	-1.3	+1.55	+1	-0.1	-1.24	-1	-0.3	+1.02	-1	-1.7	+1.33	-1
1911.....	+3.7	-.13	0	+7.3	-3.76	-5	+1.7	-2.02	-4	+12.7	-5.91	-9
1914.....	+2.5	+1.12	-4	+3.8	+6.39	+6	+2.7	+1.99	-1	+9.0	+7.50	+1
1921.....	+3.6	-.98	-3	+5.0	+1.11	-2	+2.3	-.90	+2	+10.9	-1.77	-3
1922.....	+2.1	-.86	+3	+4.0	-1.95	+2	-2.7	+2.38	+6	+3.4	-.43	+11
1925.....	-1.1	-2.83	-3	+2.8	+2.32	+2	+1.7	-1.78	-1	+3.4	-2.29	-2
1926.....	+4.5	-1.13	+1	-.5	-1.74	-2	+2.1	-2.01	+1	+6.1	-4.88	0
Average.....	+2.0	-.61	0	+3.2	+.02	0	+1.1	-.33	0	+6.3	-.92	0

## LENGTH OF FRUITING PERIOD 28 TO 31 DAYS

1907.....	-5.5	-0.89	+1	-2.0	+1.93	0	-0.1	-0.19	0	-7.6	+0.85	+1
1908.....	-1.2	+1.27	+7	-2.2	+6.92	+8	-2.3	+4.23	+2	-5.7	+12.42	+17
1913.....	+1.3	+1.94	+1	+2.6	-2.05	-1	+4.5	-1.90	-1	+8.4	-2.01	+1
1917.....	-4.9	-.09	0	-1.7	+1.71	+3	+1.9	-3.29	+4	-4.7	-1.67	-1
1919.....	-1.0	-.25	-2	+1.6	+1.67	+2	+5.7	-3.47	-5	+6.3	-3.05	-5
1920.....	-.7	-.31	+3	+1.6	-2.27	-4	+1	-.05	+1	+1.0	-2.63	0
1923.....	-1.5	-.77	+1	+1.7	+1.93	+1	+2.3	-1.97	-3	+1.5	-1.81	-1
1927.....	-.5	-2.51	-2	-1.8	-.89	-1	-1	-1.97	-1	-2.4	-5.37	-4
1929.....	-1.7	-.80	-2	-1.0	-2.46	-2	+1.9	+1.47	0	-1.8	-1.79	-4
Average.....	-1.7	-.27	+1	-.2	+1.50	0	+1.4	-.79	-1	-.5	-.56	0

<sup>a</sup> Climatic data in this and succeeding tables supplied by T. A. Blair, senior meteorologist, Weather Bureau, U. S. Department of Agriculture, Lincoln, Nebr.

TABLE 3.—Departures from normal of mean temperatures, amount of precipitation, and number of rainy days for the months of May, June, and July for a 28-year period at Lincoln, Nebr.—Continued.

## LENGTH OF FRUITING PERIOD 32 TO 35 DAYS

Year	May			June			July			Total departure		
	Tem- pera- ture	Pre- cipi- tation	Rainy days	Tem- pera- ture	Pre- cipi- tation	Rainy days	Tem- pera- ture	Pre- cipi- tation	Rainy days	Tem- pera- ture	Pre- cipi- tation	Rainy days
	°F.	Inches	Num- ber	°F.	Inches	Num- ber	°F.	Inches	Num- ber	°F.	Inches	Num- ber
1905.....	-2.1	+1.03	+3	+0.2	-0.89	-1	-3.9	-0.60	+4	-5.8	-0.46	+6
1910.....	-4.5	-.47	+1	+1.6	-2.23	-5	+3	-.82	-1	-3.6	-3.52	-5
1912.....	+3.9	-3.12	-4	-3.0	-1.33	0	+2.5	-1.67	+1	+3.4	-6.12	-3
1916.....	+1.5	-.60	+1	-3.6	-1.21	-1	+5.6	-2.56	-3	+2.5	-4.37	-3
1918.....	+6.3	-1.26	-1	+5.8	-1.79	-2	+1.9	-1.34	0	+13.0	-4.39	-3
1924.....	-6.0	-1.88	0	-2.3	.00	+6	-3.6	+1.81	+2	-11.9	-1.07	+8
1928.....	+2.9	-1.33	-4	-4.6	-.35	+6	+1.5	+1.87	+4	-1.2	+.19	+6
1930.....	-.3	-.98	+1	-.2	-1.37	-3	+6.3	-2.79	-3	+5.8	-5.14	-5
Average.	+1.1	-1.08	0	+1.9	-1.15	0	+1.1	-.89	0	+3	-3.11	0

## LENGTH OF FRUITING PERIOD 36 TO 39 DAYS

	°F.	Inches	Num- ber	°F.	Inches	Num- ber	°F.	Inches	Num- ber	°F.	Inches	Num- ber
1903.....	-0.4	+6.64	+8	-5.0	-1.72	0	-1.0	-0.78	-1	-6.4	+4.14	+7
1904.....	-.9	+1.12	+2	-3.6	+1.08	+5	-3.8	+1.27	+7	-8.3	+1.47	+14
1906.....	+1.7	-2.55	-3	-1.0	-.72	-1	-4.1	+3.00	0	-3.4	-.27	-4
1915.....	-2.7	+.69	+3	-4.2	-1.29	+2	-4.8	+2.89	+10	-11.7	+3.29	+15
Average.	-.6	+1.22	+3	-3.5	-.66	+1	-3.4	+1.60	+4	-7.5	+2.16	+8
Normal.	61.7	4.08	11	71.4	4.32	11	76.5	3.85	9	-----	-----	-----

In years when the fruiting period was from 28 to 31 days, temperatures for the most part averaged somewhat below normal for May, near normal for June, and in the majority of years slightly above normal for July. In general, precipitation was below normal in May, slightly above normal in June, and below normal in July. On the whole, however, temperatures and precipitation for the three months were only slightly below normal. In 1908 there was a large excess of precipitation during all three months, and also a larger than normal number of rainy days.

When the fruiting period was from 32 to 35 days (with the exception of 1918, when temperatures were well above normal) the temperatures were near or slightly below normal for May and June and above normal for July. With few exceptions the precipitation was below normal, in fact more so than in the last-mentioned group.

In those years with a fruiting period of from 36 to 39 days, temperatures averaged below normal for all three months, while the rainfall was above normal for May and July and only slightly below for June. Here again there seems to be a tendency for high rainfall to be associated with low mean temperatures. A comparison of the data in Tables 2 and 3 reveals a relationship between the weather conditions prevailing during the fruiting period and for the 3-month period in any one year.

Lambert <sup>4</sup> points out that the years of rust epidemics in the spring wheat area were 1904, 1911, 1916, 1919, 1920, and 1923, whereas

<sup>4</sup> LAMBERT, E. B. Op. cit.

little or no rust occurred in 1907, 1909, 1910, 1912, 1913, 1915, 1918, and 1924. The years 1905, 1906, 1908, 1914, 1917, 1921, 1922, and 1925 he classifies as intermediate or indeterminate as far as rust was concerned. Nebraska records show that 1904, 1916, 1919, 1920, and 1923 (in western Nebraska) were epidemic years. The year 1927 should be classed as potentially an epidemic year, although the actual loss from stem rust in Nebraska was not large. In the remaining years, while the amount of rust varied, it was not severe enough to attract attention, and the actual losses were not estimable.

In all the years in which the fruiting period was between 24 to 27 days no estimable losses from rust occurred. With the exception of 1909, the average date of heading was May 29 and of ripening June 23. (Table 1.) While Lambert<sup>5</sup> includes 1911 as an epidemic year in the spring-wheat area, the length of the fruiting period at Lincoln was short (24 days). The plants were in head in late May and were ripe by June 23. The mean temperatures were high, and a large deficiency in moisture occurred, so that environmental conditions were also very unfavorable for rust development. As a group, these years can be classified as rust escaping, because of the short period from heading to ripening and also because the early maturity of the grain did not permit the development of many generations of urediospores, even though rust might have been present at the time of heading and optimum conditions for its development might have been at hand.

Three of the five epidemic years are included in the second group for which the fruiting period was from 28 to 31 days. In these years the mean temperatures for the period varied from 72.5° to 75.7° F., and in two years the precipitation and the number of rainy days favored rust development. In 1923 the rainfall was short, and in consequence there was not a great deal of stem rust in the vicinity of Lincoln. The wheat headed the first week in June, or shortly thereafter, and ripened the first week of July. During three other years in this group (1907, 1927, and 1929) the times of heading, maturity, and weather conditions were somewhat similar, and no epidemic occurred. In 1908, while temperatures were below normal and exceedingly high precipitation with many rainy days occurred, conditions favored the development of rust, and yet apparently very little developed. In 1908 and 1913 wheat headed early and ripened the latter part of June, while growth was abnormal in 1917 because of excessive winter killing.

The weather conditions and length of the fruiting period in 1916 did not differ in any important respect from the other years in this group (32 to 35 days fruiting period); therefore, some factor or combination of factors other than those mentioned was responsible for the epidemic in that year. With the exception of 1924 the wheat was in head by the first of June and ripened prior to the time at which wheat matured in 1916. A rather severe epidemic occurred in 1904, when there was an extended fruiting period, but with the exception of precipitation, the weather conditions in 1903 were very similar to those in 1904.

The data for the 28-year period indicate that stem rust will not assume epidemic proportions in eastern Nebraska during those years

<sup>5</sup> LAMBERT, E. B. *Op. cit.*

when winter wheat heads prior to, or during the first week in June and ripens before July 1. The length of the fruiting period does not permit many urediosporic generations,<sup>6</sup> and in most years weather conditions conducive to the rapid maturity of the wheat usually do not favor rust development. Rust epidemics have occurred during years when wheat did not head until June and matured in July, or when the length of the fruiting period was extended. There are years with extended fruiting periods, however, when the same weather conditions prevail as in years of wheat stem rust epidemic but when only small amounts of rust develop in Nebraska. Therefore, a study of the other factors influencing the development of stem rust was undertaken to determine the part played by some of these contributing factors in abetting or inhibiting rust development. The results of this study follow.

#### AMOUNT, TIME OF APPEARANCE, AND GENERAL DISSEMINATION OF INITIAL INOCULUM

Observations made during 10 years show that the amount of local inoculum near infected barberry bushes has varied from year to year, depending primarily on the number of viable teliospores present, the weather conditions favoring their germination, and the subsequent infection of the barberries. Furthermore, in many instances, other races of stem rust have been found in the vicinity of infected barberry bushes. On the whole the limited amount of local initial inoculum near infected barberry bushes is decreasing as barberry eradication is carried on. This is especially true when the amount is contrasted with the widespread regional distribution of wind-blown urediospores from the South.

It is difficult to determine the amount of initial inoculum which reaches Nebraska each season. During certain years primary uredia are very hard to find, even after a search of several hours in wheat fields, whereas in other years they are not only readily found in any field but in large amounts over a wide area. Primary uredia were much more readily found in 1922, 1923, and 1927 than in the other years. Therefore the amount of initial inoculum which reaches Nebraska as wind-blown urediospores usually in early June, varies each year and may depend on the number of urediospores which survive the winter in the South and the conditions affecting their development and dissemination.

The time of appearance of initial inoculum also varies with the season, as has already been discussed by the writer.<sup>7</sup> In Table 4 the dates of the appearance of primary uredia near Lincoln, Nebr., are listed. During 6 of the 10 years primary uredia were found between June 7 and 9. In the remaining four years uredia were found May 30, June 12, and as late as June 18 and 20. The average date for the 10 years was June 10, and the range was 21 days, a period sufficiently long to account for great differences in the development and severity of stem rust from year to year.

<sup>6</sup> "Urediosporic generations" is used here for the want of a more inclusive term. It is true that from the time a uredium sheds its first spores until new uredia are subsequently produced, no increase in the amount of rust is possible. From this time onward, however, no such period exists, since both old and new uredia shed spores continuously until they are depleted. The progressive rate of increase is enormous from a very small number of primary uredia providing environmental factors are favorable.

<sup>7</sup> PELTIER, G. L. Op. cit.

By means of simultaneous surveys primary uredia have been found in occasional years at about the same time in south and north-east Nebraska. As a rule, however, primary uredia have been found from a few days to a week or more later in northeast Nebraska, and from one to two weeks later in the western part of the State. Thus, the amount and time of appearance of initial inoculum together with its general distribution vary from year to year. The exact amount of initial inoculum present in Nebraska each year, however, remains one of the undetermined factors.

TABLE 4.—*Weather conditions prevailing for a 2-week period prior to the appearance of primary uredia at Lincoln, Nebr., 1921 to 1930*

Year	Date of first uredia	Beginning of 2-week period	Mean temperature for period	Amount of precipitation for period	Rainy days for period
			° F.	Inches	Number
1921.....	June 8	May 25	71.7	3.94	6
1922.....	May 30	May 16	64.4	2.36	8
1923.....	June 7	May 24	69.6	4.70	5
1924.....	June 20	June 6	70.9	2.24	10
1925.....	June 12	May 29	75.3	1.41	6
1926.....	June 8	May 25	71.0	1.61	4
1927.....	..do.	..do.	63.0	1.16	5
1928.....	June 18	June 4	67.2	1.23	6
1929.....	June 9	May 26	66.1	3.36	7
1930.....	..do.	..do.	64.9	.79	4

#### WEATHER CONDITIONS FAVORING PRIMARY INFECTION

It has been pointed out that the time of appearance of primary uredia varies from year to year at Lincoln. In Table 4 the annual climatic data for two weeks prior to the finding of primary uredia are given. The weather conditions are an additional factor that varies from year to year. From the temperature standpoint, favorable conditions for a heavy primary infection occurred in 1921, 1923, 1924, 1926, and 1928. Temperatures slightly below the optimum prevailed in 1922, 1927, 1929, and 1930. The temperature in 1925 was too high for optimum infection. Sufficient moisture and rainy days occurred during all the years included in this study, with the possible exception of 1926 and 1930, for favorable infection. Apparently temperatures suitable for primary infection are more of a limiting factor than is moisture, in most years in Nebraska.

#### RELATION OF THE TIME OF HEADING OF TURKEY RED WHEAT TO DATE OF APPEARANCE OF PRIMARY UREDIA AT LINCOLN

Table 5 shows that the appearance of primary uredia does not always coincide with the time of heading of winter wheat. In 1922, 1923, and 1927 primary uredia were found at about the time of heading, whereas in 1921, 1924, 1925, 1928, and 1930 uredia did not appear until about two weeks or more after the plants had headed. In the remaining two years rust did not appear until 6 and 11 days after heading. Thus the time of heading and the date of appearance of primary uredia have a direct bearing on the further development of rust. For example, in 1921, 1924, 1925, 1926, and 1928 only about

two weeks remained for further rust development. It must be borne in mind that wheat plants approaching maturity can not be infected with rust, so that the period of susceptibility to rust is really shorter than two weeks. In the other years from three weeks to four weeks remained for secondary and succeeding infections. In other words, in these years the time necessary for increasing urediosporic generations to a point sufficient to produce an epidemic was sufficient, provided weather conditions favorable for optimum infection and development of rust prevailed.

TABLE 5.—*Relation of the time of heading of winter wheat to the date of appearance of primary uredia at Lincoln, Nebr., 1921 to 1930*

Year	Date of heading	Date of first uredia	Days from heading to first uredia	Length of the fruiting period	Days from first uredia to ripening
			<i>Number</i>	<i>Days</i>	<i>Number</i>
1921.....	May 26	June 8	13	25	12
1922.....	May 28	May 30	2	27	25
1923.....	June 6	June 7	1	30	29
1924.....	June 3	June 20	17	33	16
1925.....	May 29	June 12	14	25	11
1926.....	May 28	June 8	11	27	16
1927.....	June 7	...do....	1	29	28
1928.....	May 29	June 18	20	35	15
1929.....	June 3	June 9	6	30	24
1930.....	May 27	...do....	13	34	21

#### WEATHER CONDITIONS FAVORING THE DEVELOPMENT OF SECONDARY AND SUCCEEDING GENERATIONS OF UREDIOSPORES

It has been shown previously that the factors responsible for the development of secondary and succeeding infections are the length of the period from the appearance of primary uredia to ripening of the grain and optimum weather conditions favoring infection and rapid development of urediosporic generations.<sup>8</sup> It has been pointed out that in five years (1921, 1924, 1925, 1926, and 1928) this period averaged two weeks and was sufficiently short to produce not more than one or possibly two generations of urediospores under optimum weather conditions. In Table 6 the weather conditions prevailing during this interval are listed. Temperatures above the optimum for infection occurred in 1921 and 1925. In the other three years while temperatures slightly below the optimum prevailed, they were more favorable for rust development, although the period from initial infection to mature urediospores would be somewhat longer. On the other hand, precipitation necessary for infection was sufficient in all the five years except 1921, so that in the main the absence of rust development was due to the shortness of the period rather than to any specific environmental factor.

<sup>8</sup> Occasionally areas of heavier infection farther south abet in producing local rust epidemics. After several days of high southerly winds, followed by rains, a heavy rust infection sometimes develops in areas where primary uredia are few and widely scattered.

TABLE 6.—*Weather conditions prevailing for secondary and succeeding infections from the appearance of primary uredia to ripening of winter wheat at Lincoln, Nebr., 1921 to 1930*

Year	Date of first uredia	Date of ripening	Days in period	Mean temperature for period	Amount of precipitation for period	Rainy days
			Number	°F.	Inches	Number
1921.....	June 8	June 20	12	78.8	0.46	3
1922.....	May 30	June 24	25	74.9	1.20	7
1923.....	June 7	July 6	29	72.8	.79	8
1924.....	June 20	do.....	16	68.4	1.93	5
1925.....	June 12	June 23	11	75.9	4.74	6
1926.....	June 8	June 24	16	70.6	2.12	6
1927.....	do.....	July 6	28	71.8	3.03	7
1928.....	June 18	July 3	15	70.3	1.92	8
1929.....	June 9	do.....	24	74.1	.44	6
1930.....	do.....	June 30	21	73.2	2.60	5

During 1922, 1923, 1927, 1929, and 1930 the period from primary uredia to ripening of the grain was three to four weeks and hence was sufficiently long for several urediosporic generations to develop. In 1922, 1929, and 1930 relatively little secondary rust developed, and only a trace could be found in the wheat fields because of hot, dry weather. The year 1929 was deficient in rainfall for the entire period, whereas in 1922 most of the rainfall occurred after the wheat was maturing. In 1930 about one-half of the total rainfall for the period occurred in one day, five days after primary uredia made their appearance. Temperatures were more favorable for optimum infection in 1923 and 1927. Most of the rain fell during two days in 1927, about the middle of June, so that there was no moisture for further development of rust later in the period. In 1923, although the precipitation was light, it was scattered over a number of days. A trace of rain was also recorded for eight days in this period. Had there been more rainfall during this period in 1923 at Lincoln, much more rust would have occurred, and an epidemic as severe as that which took place in western Nebraska would have developed. During the 10 years there was no estimable loss from rust at Lincoln. The reasons vary with the years. In 5 of the 10 years the interval between the appearance of primary uredia and the ripening of the wheat was too short, and in 3 years this interval was long (more than three weeks), but hot, dry weather prevailed. In 1927 the development of uredia was inhibited by uneven rainfall over the latter part of the interval. In 1923 the amount of precipitation at any one time during the interval was too slight. In other words, rust did not assume epidemic proportions at Lincoln in any of the 10 years under discussion, although more rust occurred in 1922, 1923, and 1927 due in part to a greater amount of initial inoculum than in the other years under discussion. It is quite apparent that the limiting factor in the development of secondary and succeeding generations of urediospores, considering only the environmental factors, is the even distribution of an above normal precipitation.



## RELATION OF ENVIRONMENTAL FACTORS TO THE DEVELOPMENT OF STEM RUST IN NEBRASKA

Up to this point the discussion of the conditions favoring rust development has centered about the data collected at Lincoln, which can not be considered typical of the whole State. In order that the size of Nebraska can be visualized, the following statement can be given: If the State were pivoted at the southeast corner and moved in a 90° angle to the south, the western part would lie in Texas; and when pivoted 90° to the north, it would lie in Minnesota. The altitude increases at the rate of 1 foot for every 7 miles westward across the State, so that, with the exception of precipitation, conditions in the spring-wheat area in northwest Nebraska are somewhat similar to those in the spring-wheat area in Minnesota and the Dakotas.

For comparison, two points were chosen as representative of central and western Nebraska, namely, North Platte and Scottsbluff. At the North Platte substation, Turkey Red winter wheat heads, on the average, about 4 days later, ripens about 7 days later, and has a fruiting period 3 days longer than at Lincoln. The earliest date of heading was May 26 (1925) and the latest June 12 (1929), a range of 17 days. The earliest date of ripening was June 29 (1922 and 1925), and the latest July 18 (1929), a range of 19 days. The length of the fruiting period ranged from 26 days (1927) to 44 days (1928), a difference of 18 days, the average being 33 days for the 10-year period.<sup>9</sup>

On the average, primary uredia appear at North Platte about 10 days later than at Lincoln, the earliest date being June 12 and the latest June 28, a difference of 16 days. The average date of the appearance of primary uredia is June 20. On the whole, the range is not so great as at Lincoln. The number of days from heading to the appearance of primary uredia ranged from 6 to 29, and from primary uredia to ripening from 8 to 30. At North Platte, conditions vary as much as if not more than at Lincoln.

Unfortunately, no data on the length of the fruiting period of winter wheat are available at the substation near Scottsbluff. However, there would be less variation between Scottsbluff and North Platte than between North Platte and Lincoln. During seven years' observations, primary uredia appeared on the average of only two days later than at North Platte, the earliest date being June 17 and the latest June 28. In Table 7, the departures from normal at Lincoln, North Platte, Scottsbluff, and of the whole State, of mean monthly temperatures, precipitation, and rainy days for the months of May, June, and July are given.

It has previously been mentioned that a rust epidemic occurred in Nebraska in 1923, and that it was especially severe in the western half of the State. At North Platte temperatures were above normal during June and July, while precipitation was above normal for May, June, and July. Similar conditions prevailed at Scottsbluff, with even greater departures from the normal. Thus, while conditions were favorable for rust development in eastern Nebraska for only a short period after a large number of primary uredia appeared, conditions were much more favorable in central Nebraska and still more so in western Nebraska, especially in the spring-wheat section.

<sup>9</sup> Data supplied by L. L. Zook, associate agronomist, Division of Dry Land Agriculture, U. S. Department of Agriculture, North Platte, Nebr.

TABLE 7.—Departures from normal of the mean temperatures, precipitation, and number of rainy days from May to July at Lincoln, North Platte, and Scottsbluff, Nebr., and for Nebraska, 1921–1930

MAY												
Year	Lincoln			North Platte			Scottsbluff			Nebraska		
	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days
	° F.	Inches	Number	° F.	Inches	Number	° F.	Inches	Number	° F.	Inches	Number
1921.....	+3.6	-0.98	-3	+2.3	-0.89	+1	+0.3	-0.78	0	+1.6	+0.09	0
1922.....	+2.1	- .86	+3	+ .8	- .25	-2	+1.6	+ .75	-1	+1.2	- .76	-1
1923.....	-1.5	- .77	+1	-1.5	+1.30	-1	+ .8	+ .46	-1	-1.6	+1.46	+2
1924.....	-6.0	-1.88	0	-5.5	- .52	0	-3.5	- .83	+2	-5.5	-1.63	-2
1925.....	-1.1	-2.83	-3	+ .7	- .86	-4	+3.9	- .27	+1	- .4	-1.47	-3
1926.....	+4.5	-1.13	+1	+5.9	-1.87	+1	+4.5	- .61	+3	+5.0	- .75	+1
1927.....	- .5	-2.51	-2	+2.7	-1.38	0	+1.2	- .20	+2	- .1	-1.05	-1
1928.....	+2.9	-1.33	-4	+3.8	+ .84	+2	+5.4	- .42	-5	+3.1	- .04	0
1929.....	-1.7	- .80	-2	-1.2	- .20	-1	- .2	-1.59	-1	-1.7	- .80	-2
1930.....	- .3	- .98	+1	-1.9	+3.32	+2	-1.2	+1.25	+4	-1.7	+1.57	+1
Normal..	61.7	4.08	11	58.7	2.78	11	55.8	2.79	10	59.1	3.53	9

JUNE												
Year	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days
	° F.	Inches	Number	° F.	Inches	Number	° F.	Inches	Number	° F.	Inches	Number
1921.....	+5.0	+0.11	-2	+4.3	-1.83	-2	+3.5	-1.07	+2	+4.0	-1.43	-2
1922.....	+4.0	-1.95	+2	+5.3	-2.35	-5	+4.0	- .64	-1	+3.2	-1.37	-3
1923.....	+ .7	+ .93	+1	+1.7	+ .93	0	+ .3	+1.30	+2	+ .3	+ .92	+1
1924.....	-2.3	.00	+6	- .1	-1.32	-1	+ .9	-1.88	-3	-1.9	+ .49	+2
1925.....	+2.8	+2.32	+2	+4.2	-1.76	-2	+1.0	+1.53	+1	+1.9	+ .68	+1
1926.....	- .5	-1.74	-2	+ .5	+ .58	+4	- .2	+1.11	+2	- .7	-1.03	-1
1927.....	-1.8	- .89	-1	-1.1	+1.29	+1	-1.6	+1.42	+5	-2.2	- .12	+1
1928.....	-4.6	- .35	+6	-4.7	+1.42	+9	-6.0	+1.16	+6	-6.0	+ .85	+5
1929.....	-1.0	-2.46	-2	+1.1	-1.98	0	- .3	+ .06	-2	-1.4	- .47	-1
1930.....	- .2	-1.37	-3	+ .9	+ .84	+6	+1.6	-1.26	-2	- .8	- .56	-2
Normal..	71.4	4.32	11	67.5	3.22	10	66.4	2.54	9	69.0	3.78	9

JULY												
Year	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days	Mean temperature	Precipitation	Rainy days
	° F.	Inches	Number	° F.	Inches	Number	° F.	Inches	Number	° F.	Inches	Number
1921.....	+2.3	+0.90	+2	+3.9	-1.94	-3	+2.5	-1.05	-2	+1.7	+0.74	+1
1922.....	-2.7	+2.38	+6	- .2	+2.17	+3	-1.3	+ .68	+1	-2.1	+1.20	+3
1923.....	+2.3	-1.97	-3	+3.0	- .89	-2	+2.2	+1.14	+2	+1.8	- .51	0
1924.....	-3.6	+ .81	+2	- .3	-1.74	-3	- .7	-1.53	0	-3.0	+ .41	+1
1925.....	+1.7	-1.78	-1	+3.3	-1.85	-3	+1.0	- .18	0	+ .8	-1.18	0
1926.....	+2.1	-2.01	+1	+2.9	- .06	+3	- .1	- .09	0	+1.0	- .59	+1
1927.....	- .1	-1.97	-1	+1.0	-2.31	0	-1.7	- .73	0	-1.3	-1.40	-1
1928.....	+ .5	+1.87	+4	+ .5	+2.30	+5	- .3	+1.41	+5	- .7	+ .65	+2
1929.....	+ .9	+1.47	0	+4.7	- .68	+2	+3.0	- .23	-3	+1.6	- .17	0
1930.....	+6.3	-2.79	-3	+5.2	-2.14	-3	+3.3	-1.55	-3	+4.1	-1.84	-3
Normal..	76.5	3.85	9	72.9	2.74	9	72.5	2.02	7	74.7	3.34	7

The year 1927 was potentially an epidemic year, since a large amount of initial inoculum reached Nebraska, and large numbers of primary uredia developed under favorable conditions. The lack of sufficient rainfall in July inhibited the development of secondary and succeeding generations of uredia over the whole State. In the remaining years, as at Lincoln, it was found that either mean temperatures were above normal, which in combination with a deficiency of rainfall (1921, 1922, 1925, 1926, 1929, 1930) inhibited the development of rust, or in occasional years an excess of precipitation (1928) was at hand, but mean temperatures were too low, or both temperatures and moisture were below normal (1924). In fact the right combination of factors favoring rust development in the western half of Nebraska is less likely to occur since mean temperatures are usually lower and the amount of precipitation is decidedly less than in eastern Nebraska. This can be seen by a comparison of normal temperatures and precipitation at the points listed in Table 7. Rust epidemics in central and

western Nebraska are possible, other factors being favorable, only when temperatures are above normal for June and July and an even distribution of rainfall well above normal prevails for these months.

### DISCUSSION

In Table 8 is a summary of some of the factors that have influenced the development of stem rust in Nebraska over a 10-year period. Weather plays a dominant rôle, since many other factors are dependent on it, at least in the different periods. The weather largely determines the time of heading and of ripening, and the length of the fruiting period of winter wheat. These are also important factors in the development of rust, once initial infection takes place. Each factor contributing to optimum rust development is influenced by a definite set of weather conditions, which are not necessarily the same for any other factor during the same or different seasons.

It has been pointed out that not only does initial inoculum appear at different times in June, in Nebraska, but the number and distribution of primary uredia vary from year to year. This variation is influenced (1) by the number and distribution of overwintering urediospores in Texas, (2) by the weather conditions affecting the increasing of a large amount of inoculum, which may progressively infect wheat to the northward, (3) by the receptiveness of wheat to infection when wind-blown urediospores reach Nebraska, and (4) by the prevailing environmental factors favoring the infection of the host. In Nebraska, no primary uredia have been found on winter-wheat plants, in the spring, prior to the late-boot or heading stage. While wind-blown urediospores may reach the State before the plant reaches this stage of development, infection does not take place because temperatures too low for infection usually prevail at this time.

In 1922, for example, a large amount of initial inoculum reached Nebraska early. Many primary uredia were found at Lincoln on May 30, at the time wheat was heading. A potential rust epidemic was in the making.

TABLE 8.—Summary of factors influencing the development of stem rust, during a 10-year period, at Lincoln, Nebr., 1921-1930

Year	Time of heading	Time of first uredia	Time of ripening	Length of fruiting period	Interval from heading to first uredia	Interval from first uredia to ripening	Weather conditions from first uredia to ripening		
	Early Intermediate Late	Early Intermediate Late	Early Intermediate Late	Short Intermediate Long	Short Intermediate Long	Short Intermediate Long	Mean temperature	Amount of precipitation	Number of rainy days
1921.....	x								
1922.....	x	x	x	x	x	x	x	x	x
1923.....		x	x						
1924.....				x					
1925.....	x								
1926.....	x	x	x	x	x	x	x	x	x
1927.....		x							
1928.....	x								
1929.....	x	x	x	x	x	x	x	x	x
1930.....	x								

Shortly thereafter, hot, dry weather accompanied by hot winds set in, which not only hastened the maturity of the wheat but also inhibited the rapid development of rust. Consequently the losses from rust during 1922 were negligible. Somewhat comparable conditions occurred in 1923 and 1927. In both years normal temperatures prevailed for optimum infection after primary uredia were found. Moisture, however, was deficient in eastern Nebraska in 1923, but in western Nebraska where temperatures and rainfall were above normal a severe rust epidemic occurred. In 1927 a deficiency in rainfall during late June and early July inhibited the further development of stem rust over the whole State.

On the other hand, only a small amount of initial inoculum reached Nebraska in 1924 and 1928. Primary uredia were not found until long after the plants were headed, and although the wheat ripened late, mean temperatures were too low for the rapid development of many generations of urediospores, so that only a trace of wheat stem rust occurred in Nebraska in these years. Thus in 1922 an epidemic was averted after a large amount of inoculum reached the State during the hot, dry weather, whereas in 1924 and 1928 the small amount of initial inoculum arrived too late to cause an epidemic. In general, it may be stated that temperature is the limiting factor for primary infection, whereas moisture is usually the limiting factor for secondary and succeeding infections in Nebraska.

A stem-rust epidemic is possible only with the following combination of factors: (1) A large amount of initial inoculum reaching Nebraska at a time when environmental factors favor maximum infection; (2) wheat entering the heading stage the first week in June or thereafter with primary uredia appearing at about this same time; and (3) an extended fruiting period during which temperature and moisture favorable for the development of many urediosporic generations prevail.

#### SUMMARY

A study of the time and length of the fruiting period of wheat in a 28-year period shows that stem-rust epidemics did not occur in eastern Nebraska when winter wheat headed prior to, or during the first week in June and ripened before the first of July. The short fruiting period prevented the development of many urediosporic generations, and the environmental factors conducive to early maturity of winter wheat usually inhibited the rapid development of stem rust. These years may be classed as rust escaping. Rust epidemics occurred during occasional years within this period but were not correlated in all instances with an extended fruiting period because during other years with the same length of the fruiting period little or no rust developed.

An intensive study, over a 10-year period, of a number of factors influencing the development of stem rust leads to the conclusion that a rust epidemic is possible in the winter-wheat area of Nebraska only when a certain combination of factors is present in sequence. These factors are: (1) A large amount of initial inoculum reaching Nebraska when conditions favor maximum infection and the subsequent production of large numbers of primary uredia over a wide area; (2) winter wheat entering the heading stage the first week of June or thereafter, with primary uredia appearing at about the same time;

and (3) an extended fruiting period, during which optimum temperature, an evenly distributed precipitation above the normal, and other favorable conditions are at hand for the rapid development of urediosporic generations.

Apparently low temperatures are the major limiting factor in the development of primary infection and subsequent development of uredia, whereas the lack of an even distribution of sufficient precipitation is the major inhibiting factor in the development of subsequent urediosporic generations during most years.

Fortunately the sequence of factors necessary for stem-rust epidemics are fulfilled only in occasional years in the winter-wheat area of Nebraska. They may occur, however, in two successive years as in 1919 and 1920, or at intervals of as long as 12 years as in 1904 and 1916, with none between.



# INHERITANCE OF DWARFING IN WHEAT <sup>1</sup>

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## INTRODUCTION

Wheat crosses are made primarily to obtain new varieties of greater economic value. Frequently, however, in the process of breeding for new varieties it is possible to obtain valuable genetic data at little extra cost. This secondary phase of plant breeding, that of furnishing a genetic interpretation for the inheritance of a character, forms the basis of the present discussion.

Certain wheat crosses, involving normally tall varieties, will give rise to dwarf plants in the progeny. This peculiar characteristic is not common to wheat alone, but, according to Waldron (12),<sup>3</sup> has been reported in the breeding of other crop plants such as maize, barley, oats, peas, beans, squash, and tomatoes. However, not all dwarfs are alike; apparently there are different types even within the same species.

## REVIEW OF LITERATURE

The dwarf character in wheat usually does not appear until the  $F_2$  generation, although some workers have observed dwarfs in the  $F_1$  (2, 7, 9).

The presence of dwarfs in the  $F_2$  and later generations has usually been explained on the basis of a 2-factor difference—one, a dominant dwarfing factor, the other an inhibiting factor. Clark and Hooker (1) in a Marquis  $\times$  Hard Federation cross, Stephens (10) in a Federation  $\times$  Master cross, and Stewart and Tingey (11) in a Federation  $\times$  Marquis cross, explained their results on the 2-factor basis. Similar explanations were given by Clark and Quisenberry (2), Hayes and Aamodt (6), and Goulden (5) for results secured in crosses involving Marquis and Kota wheats. However, from a cross involving the same varieties (Marquis and Kota), Waldron (12) reported irregular breeding behavior in respect to the dwarf and tall plants, the ratios obtained in  $F_3$  being 3:1, 13:3, 55:9, 15:1, and 63:1. These results he explained on the basis of a 3-factor difference—one factor the normal,  $N$ , and the other two complementary dwarf factors,  $A$  and  $D$ , both of which are inhibited in expression in the presence of  $N$ . Since this explanation does not account for all the ratios obtained, Waldron further concluded that the genes concerned are in a labile condition, often changing from dwarf to normal, and vice versa. In a Chul  $\times$  Marquis cross, Goulden (5) found that two factors were involved. He obtained ratios in the  $F_3$  which were difficult to explain on this

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<sup>2</sup> The writer expresses his appreciation to Mrs. Blanche C. Pittman for reading and correcting the manuscript and to Margaret Richards for calculating the data presented in the tables.

<sup>3</sup> Reference is made by number (italics) to Literature Cited, p. 94.

basis, but cytological studies gave evidence that lagging chromosomes were present in most of the cells undergoing reduction division. This, he believed, would account for the irregular breeding behavior in the  $F_3$  generation.

From a study of the proportion of dwarfs in the  $F_2$  generation of two crosses, Quality  $\times$  Jenkin and Jenkin  $\times$  Marquis, as well as of two back crosses (Quality  $\times$  Jenkin)  $\times$  Quality and (Jenkin  $\times$  Marquis)  $\times$  Marquis, Florell (4) concluded that there was a 3-factor difference between normal and dwarf. Neethling, according to Matsuura (8), reported that in tall  $\times$  tall crosses he obtained in the  $F_2$  generation 8 dwarfs in a total of 31 plants, or what was considered a 1:3 ratio. However, so few individuals are unreliable as a measure of the true ratio. Furthermore, these same data, if any reliance can be placed on results obtained with so few plants, are within the limits of the error, when considered on a 13:3 basis. If the study had been con-

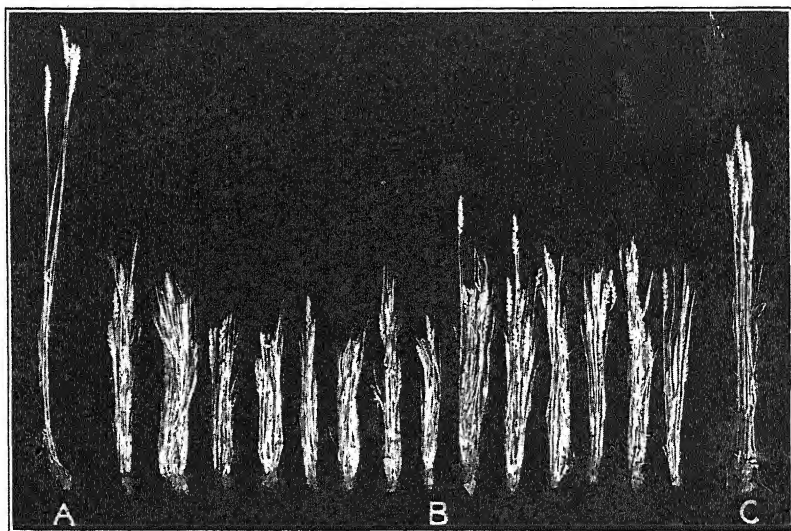


FIGURE 1.—Homozygous dwarf plants (B) in comparison with the two parents, Dicklow (A) and Hard Federation (C)

tinued into the  $F_3$  generation, the question as to whether the true ratio is 3:1 or 13:3 would have been answered.

#### EXPERIMENTAL METHODS AND MATERIAL

Crosses were made and carried through the different generations in the usual way. Pollen from the anthers of a single spike was used to pollinate the stigmas of a single spike. All seedlings were made in the field. The genetic composition of the  $F_2$  generation was determined by the breeding behavior of the  $F_3$ . In a number of crosses height data were taken on the  $F_2$  plants, and a biometrical study was made of these data. The  $F_3$  generation usually consisted of 200 or more rows except in some of the tall  $\times$  dwarf crosses, where this number was not available. Each  $F_3$  row was seeded from a single  $F_2$  plant. The number of  $F_3$  rows is shown in the various goodness-of-fit tables. The  $F_3$  rows were usually 12 feet long and the kernels were spaced from 2 to 3 inches apart. This gave from 25 to 70 mature plants to the row.



In studying the goodness of fit, the  $\chi^2$  test as given by Fisher (3) was used.

The dwarf plants referred to in this paper were somewhat variable in height, leafiness, and time of maturity. In these respects they were similar to tall varieties. Some dwarfs were short, grasslike clumps with few culms, which matured little, if any, seed; others were short but not so grassy and matured considerable seed when grown under favorable conditions; still others were rather grassy

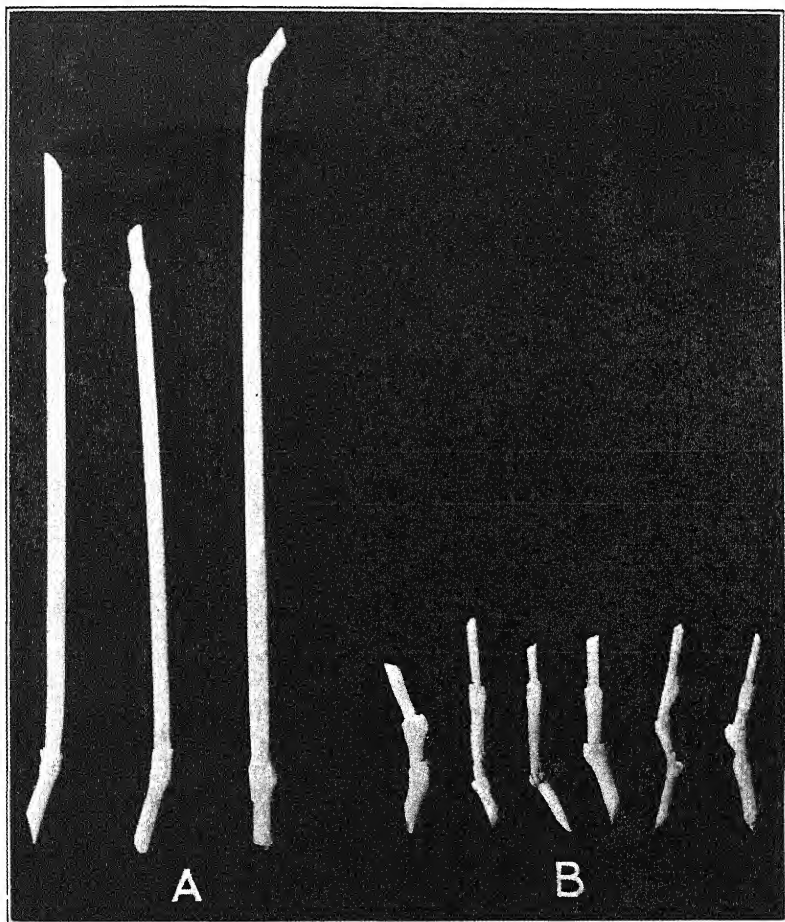


FIGURE 2.—Relative length of the lower second or third internode in tall (A) and dwarf (B) plants

clumps with one or two culms nearly as long as those of the tall varieties. The last-mentioned type is usually heterozygous. In Figure 1 are shown some dwarfs from a homozygous row in comparison with the tall parents.

The dwarfing characteristic is the result of a shortening of the second or third internode near the base of the plant; sometimes both internodes are so shortened that the nodes appear to be almost together. (Fig. 2.) The upper internodes are somewhat shorter in dwarfs than in tall plants, but less markedly so than the lower.

## EXPERIMENTAL DATA

The data reported were obtained from a study of 92 different crosses involving some 50 or more strains and varieties of wheat. Detailed genetic studies, however, have been made of only a comparatively few of these 92 crosses. A number of varieties have been intercrossed in such a way as to provide an additional test of the breeding behavior of the character under consideration. The genetic composition of strains and varieties has been studied in three different ways: (1) By the usual method of studying the breeding behavior in the  $F_2$  and  $F_3$  generations involving tall  $\times$  tall crosses; (2) by studying the breeding behavior in the  $F_2$  and  $F_3$  generations with tall  $\times$  dwarf crosses; and (3) by intercrossing varieties in different combinations.

TALL  $\times$  TALL CROSSES

Of the crosses in which both parents were tall, 36 gave dwarfs in  $F_2$ . Eleven of these were studied. In 4 of the 11 the genetic composition of the  $F_2$  dwarfs was determined by the  $F_3$  breeding behavior, but in the remaining 7 only the tall  $F_2$  plants were seeded for study of the  $F_3$ . These 7 crosses involved winter wheats. It was difficult to secure seed from dwarf plants grown under dry-land conditions, and for this reason it was not possible to continue the study of the dwarfs into the  $F_3$  generation.

TABLE 1.—Classification of  $F_2$  plants into tall and dwarf and the calculated  $\chi^2$  test for goodness of fit based on a 13:3 ratio

Cross No.	Parents	Class	Observed	Calculated	$\chi^2$	P
23a	Dicklow $\times$ No. 4 Sevier No. 121.....	{Tall..... {Dwarf.....	407 86	400.6 92.4	}.4535	0.50-0.70
35c	Dicklow No. 3 $\times$ F-22 (Dicklow $\times$ Sevier).....	{Tall..... {Dwarf.....	212 45	208.8 48.2		
39a	Dicklow No. 3 $\times$ Hard Federation.....	{Tall..... {Dwarf.....	256 55	252.7 58.3	}.2299	.50- .70
47e	Marquis $\times$ Hard Federation.....	{Tall..... {Dwarf.....	216 51	216.9 50.1		

## SPRING-WHEAT CROSSES

 $F_2$  DATA

Seed from the dwarfs in the spring-wheat crosses grown under irrigation generally germinated well if planted reasonably early. Height measurements were taken on the  $F_2$  plants in the four spring-wheat crosses involving tall parents, and the plants were classified as tall and dwarf. The results of this classification are shown in Table 1. In Table 1 the data are fitted to a 13:3 ratio. The assumption is that the contrasted characters, tall and dwarf, differ by two genetic factors—a dominant dwarfing factor (*DD*) and an inhibiting factor (*II*). When two varieties are crossed and dwarfs occur in the  $F_2$  generation, it is thought that one parent must carry both dominant factors, *DDII*, and the other must carry both recessive factors, *ddii*. When these two types are crossed and the  $F_1$  is selfed the theoretical genetic composition and breeding relationships shown in Table 2 should be obtained. This table indicates that theoretically at least the  $F_2$  phenotypic ratio should be 13 tall:3 dwarf. If this be true, a similar segregating ratio would be expected when certain tall varieties are crossed. (Fig. 3.)

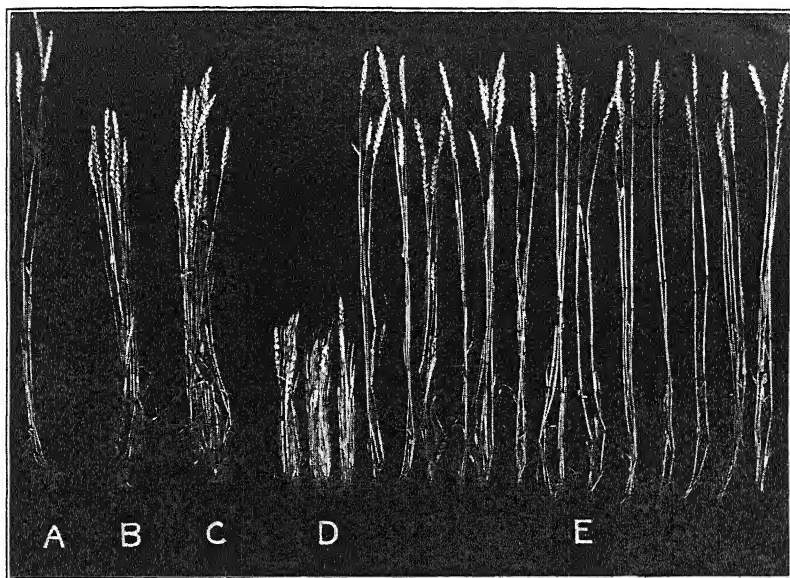


FIGURE 3.—Results in  $F_1$  and  $F_2$  from a tall ( $ddii$ )  $\times$  tall ( $DDII$ ) cross: A, Dicklow parent ( $ddii$ ); B, Hard Federation parent ( $DDII$ ); C,  $F_1$  tall ( $DdIi$ ); D,  $F_2$  dwarf, 3; E,  $F_2$ , tall, 13

TABLE 2.—Theoretical breeding behavior based on a 2-factor difference, one a dominant dwarfing factor ( $DD$ ) and the other an inhibiting factor ( $II$ )

Zygotes:  $DDII$  (tall)  $\times$   $ddii$  (tall);  $F_1$ ,  $DdIi$  (tall)]

$F_2$ phenotypic ratio	Proportion of $F_2$ genotypes	Genotype	$F_3$ breeding behavior	$F_3$ breeding ratio
13 tall.....	1	$DDII$	Tall.....	7
	2	$DdII$	.....do.....	
	1	$ddII$	.....do.....	
	2	$ddIi$	.....do.....	
	1	$ddii$	.....do.....	
3 dwarf.....	4	$DdIi$	13 tall : 3 dwarf.....	4
	2	$DDII$	3 tall : 1 dwarf.....	2
	2	$Ddii$	1 tall : 3 dwarf.....	2
	1	$DDii$	Dwarf.....	1
	1	$DDii$	Dwarf.....	1
Total.....	16			16

The four crosses studied in  $F_2$  approximate this proportion of tall and dwarf plants. (Table 1.) The  $\chi^2$  test for goodness of fit shows the value of  $P$  to be large enough in all cases for the proportion of tall and dwarf to be considered well within the range of the sampling error. If  $P$  is between 0.1 and 0.9, there is no reason to suspect the hypothesis tested; and even if the value of  $P$  is as low as 0.05, the discrepancy is not serious (3).

#### $F_3$ DATA

Seed of both tall and dwarf  $F_2$  plants from each of the crosses listed in Table 1 was sown. Theoretically, if the 2-factor difference ( $DDII$  and  $ddii$ ) is the correct explanation for the inheritance of dwarfing, then the segregation in  $F_3$  should be as shown in Table 2;

that is, the  $F_3$  rows should give a ratio of 7:4:2:2:1. The 7 should all be tall; the 4 should segregate 13 tall:3 dwarf; 2 should segregate 3 tall:1 dwarf; 2 should segregate 1 tall:3 dwarf; and 1 should be true dwarf.

Ratios of 13:3 and 3:1 are so nearly alike that it would be difficult to know which was the true one without further tests, unless large numbers of individuals were available in each case. In the  $F_3$  rows, however, the number of plants is usually rather small; therefore, an attempt to classify the rows segregating 13:3 and 3:1 would be of doubtful value. It may appear that the deviates in the two ratios would tend to overlap; however, since these two ratios occur in the proportion of 4:2, an error is introduced, for the overlapping deviates would be more in one direction than in the other. In view of these complications, no attempt has been made to separate these two classes in the  $F_3$  population. When the two classes are combined, the ratio becomes 7:6:2:1 instead of 7:4:2:2:1. The goodness-of-fit tests are shown in Table 3. Here again the value of  $P$  is in all instances sufficiently large to be well within the expected limits.

TABLE 3.—Goodness of fit from  $F_3$  breeding classes based on a 7:6:2:1 ratio obtained in various crosses

Cross No.	Parents	Observed	Calculated	$\chi^2$	P
23a	Dicklow No. 4 $\times$ Sevier 121.....	210	215.7	1.4723	0.60-0.70
		197	184.9		
		56	61.6		
		30	30.8		
		107	108.5		
35c	Dicklow No. 3 $\times$ F-22 (Sevier $\times$ Dicklow).....	98	93.0	.5637	.90-.95
		29	31.0		
		14	15.5		
		135	133.4		
		116	114.4		
39a	Dicklow $\times$ Hard Federation.....	34	38.1	.5252	.90-.95
		20	19.1		
		108	116.3		
		107	99.7		
		35	33.2		
47e	Marquis $\times$ Hard Federation.....	16	16.6	1.2461	.70-.80

#### WINTER-WHEAT CROSSES

Seven of the winter-wheat crosses segregated for tall and dwarf in the  $F_2$  generation. The dwarf plants were not carried into the  $F_3$  for reasons previously mentioned. Because of this it is possible to determine only the breeding behavior of the tall plants. The proportion of tall and dwarf plants in the  $F_2$  generation in the winter-wheat crosses was extremely variable, owing possibly to the abnormal development which took place under dry-land conditions. Since the dwarf plants were usually short grassy clumps, it was difficult to determine in some cases whether the small dead plants were dwarfs or whether they were potentially tall plants that had been winter-killed. This uncertainty of classification in  $F_2$  would introduce a serious error and thus make the goodness-of-fit test unreliable, whereas in  $F_3$  it was much simpler to list true-breeding or segregating rows, since more plants were involved; consequently, the  $F_3$  data should be reliable.

F<sub>3</sub> DATA

On the basis of the data shown in Table 2, the tall F<sub>2</sub> plants should segregate in F<sub>3</sub> in the proportion of 7 (homozygous tall):6 (segregating). Fitted to this expected 7:6 ratio, the results from the seven crosses are shown in Table 4. The value of *P* is 0.1 or more in all cases. From these and the preceding results it appears that the 2-factor theory explains the inheritance of dwarfing in these crosses. Consequently, the genetic composition of the tall parents which on crossing throws dwarfs in F<sub>2</sub> are *DDII* and *ddii*.

TABLE 4.—Goodness of fit for two classes: (1) True breeding tall, (2) segregated tall and dwarf (13:3 and 3:1 classes) in F<sub>3</sub> generation based on a 7:6 ratio

Cross No.	Parents	Class	Observed	Calculated	$\chi^2$	P
67a...	Ridit × Sevier 59.....	{Tall.....	131	134.6	0.2086	0.50-0.70
		{Segregating.....	119	115.4		
Do.....	do.....	{Tall.....	157	159.9	.1139	.70-.80
		{Segregating.....	140	137.1		
69a...	Ridit × Utac.....	{Tall.....	126	129.2	.1717	.50-.70
		{Segregating.....	114	110.8		
Do.....	do.....	{Tall.....	207	212.7	.3309	.50-.70
		{Segregating.....	188	182.3		
70a...	Ridit × 14-85 (Federation × Sevier).....	{Tall.....	136	134.6	.0316	.80-.90
		{Segregating.....	114	115.4		
103a...	Federation × Martin.....	{Tall.....	151	156.2	.3752	.50-.70
		{Segregating.....	139	133.8		
107a...	Federation × Hohenheimer.....	{Tall.....	117	120.6	.2328	.50-.70
		{Segregating.....	107	103.4		

## TALL × DWARF CROSSES

Tall × dwarf crosses were made to determine more definitely whether or not the 2-factor theory is correct and also to ascertain which variety or varieties carry the dwarfing factor. These tall × dwarf crosses are listed in Table 5. Homozygous dwarfs were used as the dwarf parents. The tall parents were varieties that had been used in previous studies of tall × tall crosses.

TABLE 5.—Varieties used in the tall × dwarf crosses and type of plants in the F<sub>1</sub>

Cross No.	Female parent	Male parent	F <sub>1</sub> tall or dwarf
60.....	Dicklow No. 3.....	Q 100-4 (dwarf).....	Dwarf.
61.....	do.....	Q 114-14-3 (dwarf).....	Do.
62.....	do.....	Q 100-1 (dwarf).....	Do.
63.....	do.....	Q 114-13 (dwarf).....	Do.
64.....	do.....	Q 136-17-1 (dwarf).....	Do.
65.....	do.....	Q 100-2 (dwarf).....	Do.
96.....	Federation.....	39a-304 (dwarf).....	Tall.
97.....	Sevier No. 59.....	do.....	Do.
98.....	Hard Federation.....	do.....	Do.
99.....	Marquis.....	do.....	Dwarf.

If the tall × tall crosses segregating for tall and dwarfs in F<sub>2</sub> are *DDII* and *ddii*, then the homozygous dwarf should be *DDii*. The genetic composition and theoretical breeding behavior of these *DDII* (tall) × *DDii* (dwarf) and *ddii* (tall) × *DDii* (dwarf) are shown in Table 6. It will be noted that both of the tall types differ from the dwarf in only one factor pair. Hence, crosses with dwarfs ought to give ratios of either 3 tall:1 dwarf (or 1:2:1) or 1 tall:3 dwarf

(or 1:2:1) in  $F_2$  and a ratio of 1:2:1 in  $F_3$ . The  $F_1$  generation will show the genetic composition of the tall varieties, since the genetic type  $DDII$  (tall)  $\times$   $DDii$  (dwarf) cross will give  $DDi$ , which will be tall in  $F_1$ , whereas the genetic type  $ddii$  (tall)  $\times$   $DDii$  (dwarf) will give  $Ddii$ , which will be dwarf in  $F_1$ . (Figs. 4 and 5.)

TABLE 6.—Genetic composition and theoretical breeding behavior of tall ( $DDII$ )  $\times$  dwarf ( $DDii$ ) and tall ( $ddii$ )  $\times$  dwarf ( $DDii$ )

Parents	$F_1$	$F_2$ phenotypic ratio	$F_3$ breeding behavior
Dwarf ( $DDii$ ) $\times$ tall ( $DDII$ )	tall ( $DDIi$ )	3 tall : 1 dwarf or (1:2:1)	1 tall, 2 segregating, 1 dwarf.
Dwarf ( $DDii$ ) $\times$ tall ( $ddii$ )	dwarf ( $Ddii$ )	1 tall : 3 dwarf or (1:2:1)	1 tall, 2 segregating, 1 dwarf.

### $F_2$ DATA

The  $F_2$  data secured from studies involving two genetic types of tall crossed with dwarf are given in Table 7. It will be observed that in all cases the goodness of fit as measured by  $P$  is 0.10 or more. Figures 4 and 5 show the results of crossing each of the tall types  $DDII$  and  $ddii$  with dwarf.

TABLE 7.—Goodness of fit for two  $F_2$  phenotypes based on ratio of either 3 tall : 1 dwarf or 1 tall : 3 dwarf

Cross No.	Parents <sup>a</sup>	$F_1$	$F_2$ class	Observed	Calculated	$\chi^2$	P
60a	Dicklow $\times$ dwarf (q100-4)	Dwarf	(Tall..... Dwarf.....	9 32	10.2 30.8	0.1880	0.50-0.70
60e	do	do	(Tall..... Dwarf.....	11 36	11.7 35.3		
60g	do	do	(Tall..... Dwarf.....	15 37	13 39	.4103	.50-.70
96a	Federation $\times$ dwarf (39a-304)	Tall	(Tall..... Dwarf.....	193 63	192 64		
97a	Sevier 59 $\times$ dwarf (39a-304)	do	(Tall..... Dwarf.....	197 61	193.5 64.5	.0207	.80-.90
98	Hard Federation $\times$ dwarf (39a-304)	do	(Tall..... Dwarf.....	181 59	180 60		
99	Marquis $\times$ dwarf (39-304)	Dwarf	(Tall..... Dwarf.....	55 131	46.5 139.5	2.0717	.10-.20
63b	Dicklow $\times$ dwarf (q114-13)	do	( <sup>b</sup> )				

<sup>a</sup> Numbers in parentheses refer to dwarf number.

<sup>b</sup> Difficult to classify.

### $F_3$ DATA

Both tall and dwarf plants were seeded for a study of the  $F_3$  progeny. The data obtained were fitted to the expected ratio of 1 tall : 2 segregating : 1 dwarf. (Table 8.) It is obvious that in all crosses, except 63b, Dicklow  $\times$  Dwarf (q114-13) the observed ratios fit the expected when tested by  $\chi^2$ . The plants in the  $F_2$  generation of this cross were difficult to classify. Dominance was apparently incomplete. The  $F_2$  plants varied in length of culm from dwarf to tall. Some plants developed an unusually heavy vegetative growth. In this respect they resembled the dwarf type, yet these same plants had culms ranging in length from those characteristic of dwarfs to those characteristic of the tall varieties. Because of this difficulty, goodness-of-fit data for cross 63b in the  $F_2$  generation were not included in Table 7.

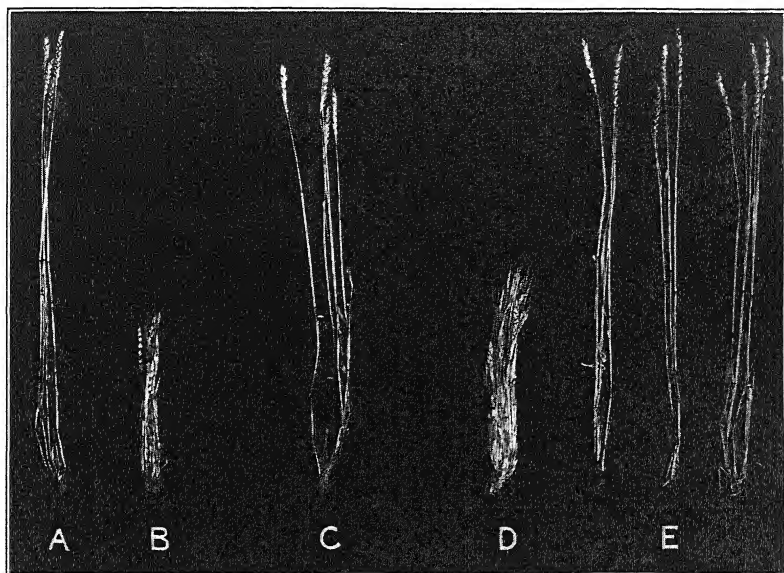


FIGURE 4.—Results in  $F_1$  and  $F_2$  from a tall ( $DDII$ )  $\times$  dwarf ( $DDii$ ): A, Hard Federation parent ( $DDII$ ); B, dwarf parent ( $DDii$ ); C, tall  $F_1$  ( $DDIi$ ); D,  $F_2$  dwarf, 1; E,  $F_2$  tall, 3. Compare with Figure 5

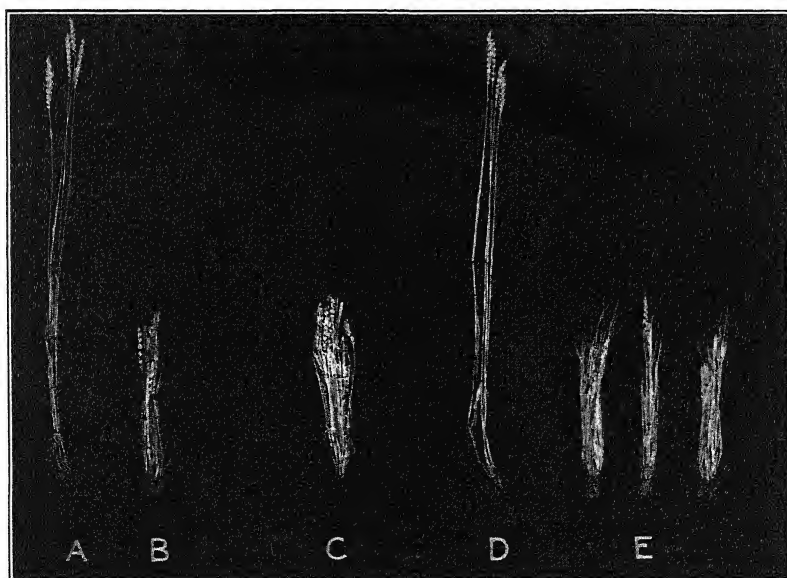


FIGURE 5.—Results in the  $F_1$  and  $F_2$  from a tall ( $ddii$ )  $\times$  dwarf ( $DDii$ ) cross: A, Dicklow parent ( $ddii$ ); B, dwarf parent ( $DDii$ ); C, dwarf  $F_1$  ( $Ddii$ ); D,  $F_2$  tall, 1; E,  $F_2$  dwarf, 3. Compare with Figure 4

TABLE 8.—Goodness of fit for three  $F_3$  breeding classes based on a 1:2:1 ratio as obtained in various crosses involving tall and dwarf types

Cross No.	Parents <sup>a</sup>	Class	Observed	Calculated	$\chi^2$	P
60a	Dicklow $\times$ dwarf (q100-4) .....	Tall.....	9	10.2	0.2161	0.80-0.90
		Segregating.....	21	20.5		
		Dwarf.....	11	10.2		
60e	do.....	Tall.....	11	11.5	.7391	.50-.70
		Segregating.....	21	23.0		
		Dwarf.....	14	11.5		
60g	do.....	Tall.....	15	13.0	1.1385	.50-.70
		Segregating.....	27	26.0		
		Dwarf.....	10	13.0		
63b	Dicklow $\times$ dwarf (q114-13) .....	Tall.....	32	31.5	16.0159	.0001-.0003
		Segregating.....	81	63.0		
		Dwarf.....	13	31.5		
96	Federation $\times$ dwarf (39a-304) .....	Tall.....	65	64.0	.0312	.95-.98
		Segregating.....	128	128.0		
		Dwarf.....	63	64.0		
97	Sevier 59 $\times$ dwarf (39a-304) .....	Tall.....	74	66.5	2.2331	.30-.50
		Segregating.....	135	133.0		
		Dwarf.....	57	66.5		
98	Hard Federation $\times$ dwarf (39a-304) .....	Tall.....	54	60.0	1.0250	.50-.70
		Segregating.....	127	120.0		
		Dwarf.....	59	60.0		
99	Marquis $\times$ dwarf (39a-304) .....	Tall.....	52	45.7	2.4780	.20-.30
		Segregating.....	81	91.5		
		Dwarf.....	50	45.7		

<sup>a</sup> Numbers in parentheses refer to dwarf numbers.

Some of these intermediate types of plants, when seeded in  $F_3$ , segregated for tall and dwarf; others bred true for tall; and still others bred true for dwarf, though the dwarfs were taller than usual. A few appeared to breed true for this intermediate characteristic. However, when plants were selected from these and seeded in  $F_4$ , some bred true for tall, others for dwarf, and still others segregated, thus showing that these intermediates were heterozygous. The true-breeding tall plants selected from these intermediate rows appeared shorter than the tall parent used in the cross. This shortening of the culm length might be traceable to Hard Federation, since this variety was one of the original parents used in the cross from which was selected the dwarf plant employed as a parent in cross 63b. There are, doubtless, factors other than the dwarfing factor which influence the length of culm, since there are varieties with different culm lengths which show no dwarfing characteristics; likewise, there are dwarfs with different culm lengths.

It is interesting to note from the goodness-of-fit studies in Table 8, that in cross 63b (Dicklow  $\times$  dwarf), the number of true-breeding tall plants was almost exactly as expected. By grouping the other two classes (the segregating and the dwarf) into one, a good fit to a 3:1 ratio is obtained. The discrepancy in this cross is due to the fact that there are too many in the segregating group and too few in the true dwarf group on the basis of a 1:2:1 ratio. This discrepancy may be accounted for by assuming that some natural crossing took place in the field among the  $F_2$  plants. This is a perfectly logical assumption, since it has been shown that in some wheats, under certain conditions, there may be a high percentage of crossing (7). Crossing occurs more often on abnormally than on normally developed spikes. Abnormally developed spikes are more or less common in dwarfs. It was on just such plants as these (dwarfs) that natural crossing is believed to have occurred. The Dicklow is known to be of the *dit*



type. The  $F_1$  plant was a dwarf and consequently should have given a ratio, in  $F_2$ , of 3 dwarfs:1 tall, or 1:2:1 if dominance was incomplete. Thus, all tall plants in  $F_2$  should breed true for tallness in  $F_3$ . Two of the  $F_2$  dwarfs should segregate in  $F_3$  and the third should breed true. It so happened, however, that a larger proportion segregated. Obviously, it could be supposed that when the  $F_2$  plants were in bloom pollen from tall plants fell on the lower dwarfs and caused fertilization. If pollen from a tall plant,  $dd$ , were to fertilize a dwarf,  $DD$ , a dwarf or intermediate plant in  $F_2$  would result. Instead, therefore, of being  $DD$  (true dwarf), it would be  $Dd$ , and consequently would segregate in  $F_3$ , thus giving a proportionately higher percentage of segregates than would have occurred under normal self-fertilization. Part of this effect would be counterbalanced if heterozygous dwarfs,  $Dd$ , were pollinated by tall plants,  $dd$ , since the proportion of dwarf and tall would be reduced from 3 dwarf:1 tall to 1 dwarf:1 tall.

It would be very difficult for the pollen from the dwarfs to fertilize the tall plants. Moreover, the spikes on the tall plants develop more normally, thus excluding the possibility of any appreciable number of tall plants being fertilized by pollen from dwarf plants; consequently, this group would behave normally, thus accounting for the close approach to the expected ratio.

#### NATURAL CROSSING BETWEEN TALL AND DWARF PLANTS

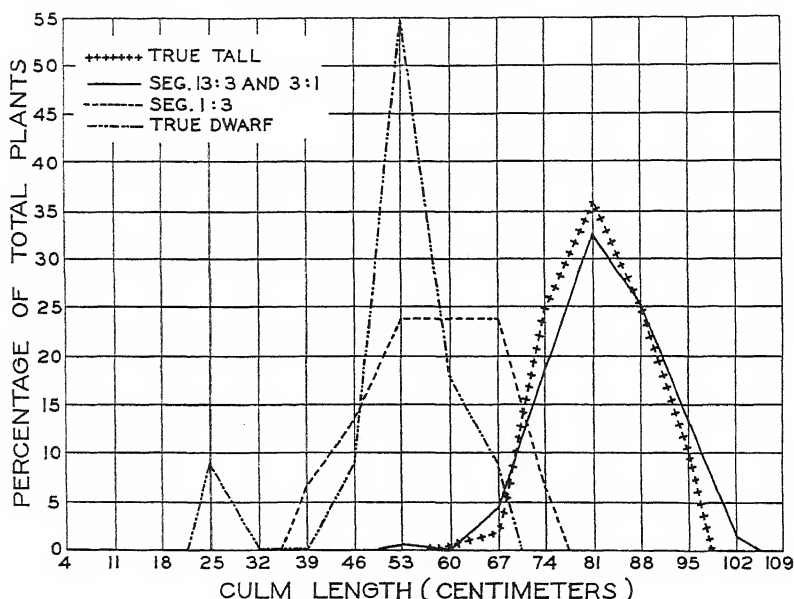
Since it was suspected that natural crossing occurred, and since this provided a logical explanation of the irregular breeding behavior of one of the tall  $\times$  dwarf crosses, it appeared desirable to determine whether or not there was natural crossing from tall to dwarf plants. In one part of the grain nursery a series of rows was sown with seed from homozygous dwarfs and from the original tall parents of the cross from which the dwarfs had been selected. The dwarf strain in one of these rows had been grown for several years and had been used in a number of tall  $\times$  dwarf crosses. Seed from this dwarf was sown about 4 feet from Hard Federation. Since Hard Federation is of the  $DDII$  type, it was possible to determine whether or not crossing occurred by simply making seedlings from plants selected from the dwarf row. This was possible because of the fact that the composition of these dwarf plants is  $DDii$  and any crossing that might occur with a  $DDII$  (tall) plant would give rise to a plant of the composition  $DDIi$ ; that is, a tall plant.

Twenty-five plants were chosen at random from the dwarf row, and seeds from each were sown the following year in single 12-foot rows. This gave about 40 mature plants to the row. A similar row was sown from 2-year-old seed of the kind sown to produce the dwarf row from which selections were made to test the amount of natural crossing with Hard Federation. Later in the season it was obvious that natural crossing had occurred, since 13 of the 25 rows, or 52 per cent, contained both tall and dwarf plants. (Table 9.) In these 25 rows the percentage of natural crossing ranged from 0 to 7.6, with an average of 1.8. The row planted from 2-year-old seed contained no tall plants.

Further evidence of natural crossing was found when a study was made of the inheritance of dwarfing in  $F_3$  rows, for in some rows one or two tall plants were observed, whereas all were expected to be dwarfs.

TABLE 9.—Natural crossing of tall (*DDII*) and dwarf (*DDii*) when grown 4 feet from each other in the field

Source of seed	Rows	Approximate plants to the row	Natural crossing		
			Rows	Range in percentage of rows	Average percentage of total plants showing natural crossing
Dwarf, 39a-304, 1930 seed.....	Number 1	Number 40	Per cent 0	0	0
Selected dwarfs from 39a-301, 1931 seed.....	25	40	52	0-7.6	1.8

FIGURE 6.—Distribution of culm length of the  $F_2$  plants for different breeding classes in  $F_3$  in the cross Dicklow 3  $\times$  F-22 (Sevier  $\times$  Dicklow). (For convenience a whole number was taken for the class center)BIOMETRICAL STUDY OF HEIGHT IN TALL AND DWARF  $F_2$  PLANTS

Height measurements were taken on the longest culm of each  $F_2$  plant in a number of crosses. These plants were later classified on the basis of the  $F_3$  breeding behavior. Statistical constants were calculated for each class. (Table 10 and figs. 6 to 10.) In the crosses Dicklow 3  $\times$  Hard Federation and Marquis  $\times$  Hard Federation, the range in dwarf plants in  $F_2$  was distinctly outside that of the tall plants. (Table 10.) The range and the mean height for the segregating or heterozygous dwarfs were not materially different from those for the homozygous. There was considerable overlapping of tall and dwarf in the Dicklow 3  $\times$  F-22 (Sevier  $\times$  Dicklow) cross. This overlapping of tall and dwarf was characteristic also of the Dicklow 3  $\times$  Hard Federation.

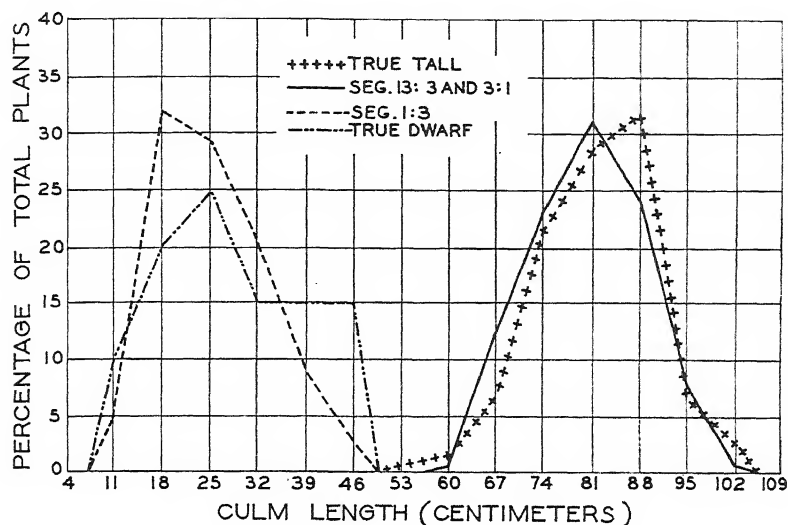


FIGURE 7.—Distribution of culm length of the F<sub>2</sub> plants for different breeding classes in F<sub>2</sub> in the cross Dicklow 3 × Hard Federation. (For convenience a whole number was taken for the class center)

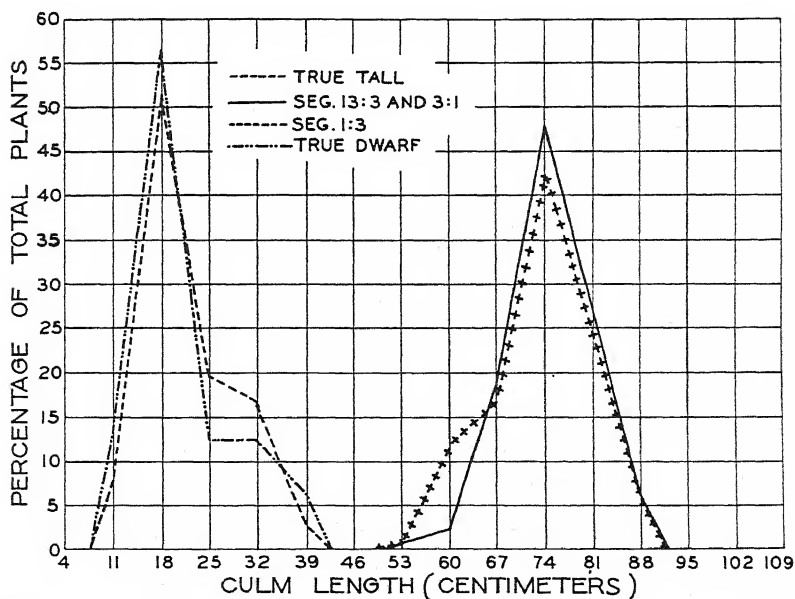


FIGURE 8.—Distribution of culm length of the F<sub>2</sub> plants for different breeding classes in F<sub>2</sub> in the cross Marquis × Hard Federation. (For convenience a whole number was taken for the class center)

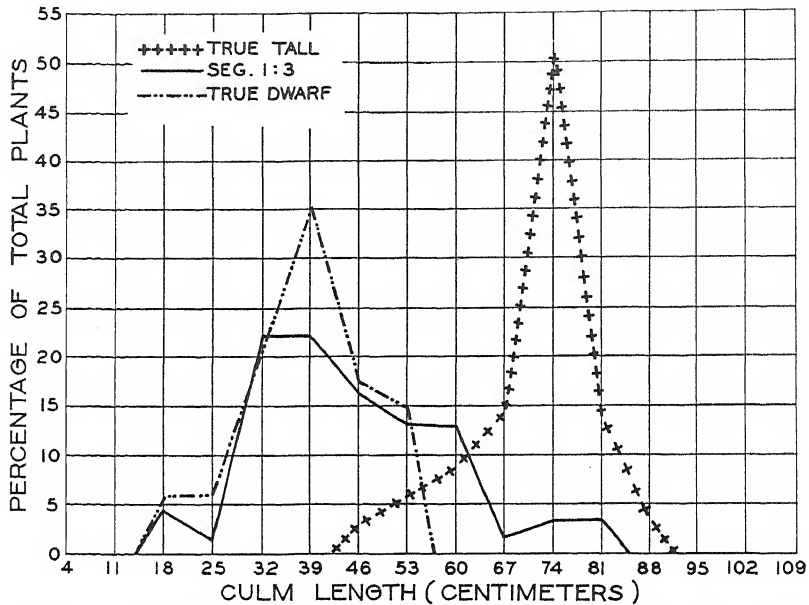


FIGURE 9.—Distribution of culm length of the F<sub>2</sub> plants for different breeding classes in F<sub>3</sub> in the cross Dicklow X Dwarf (q100-4). (For convenience a whole number was taken for the class center)

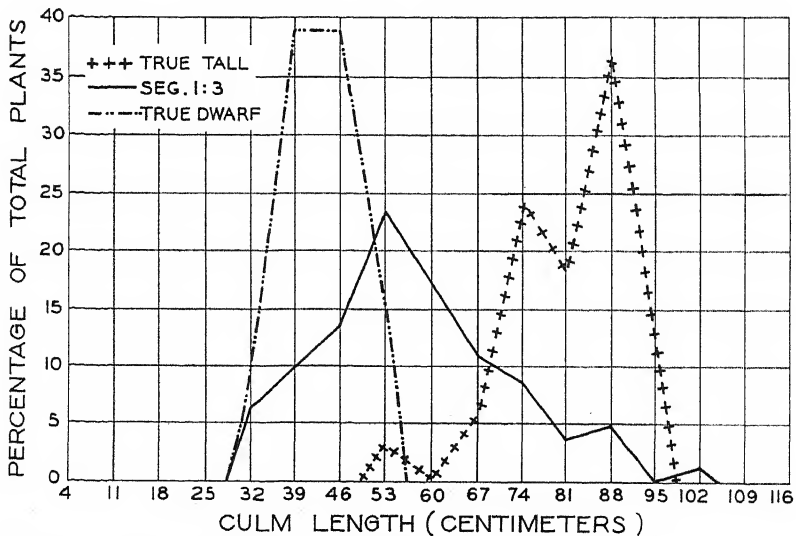


FIGURE 10.—Distribution of culm length of the F<sub>2</sub> plants for different breeding classes in F<sub>3</sub> in the cross Dicklow X Dwarf (q114-13). (For convenience a whole number was taken for the class center)

TABLE 10.—Frequency distribution for the height of the  $F_2$  plants for various breeding classes in  $F_3$

[illegible]

Dwarf plants are considerably more variable than tall plants, as is shown by the coefficient of variability. Crosses 69a and 70a were both grown on the dry farm; these plants show less variability than spring-planted crosses grown under irrigation.

#### INTERCROSSING VARIETIES AND THEIR BREEDING BEHAVIOR WITH RESPECT TO DWARFING

In regard to dwarfing in wheat it may be concluded that the 2-factor theory provides a logical explanation for the inheritance of this character. Obviously there are two types of tall varieties: (1) When tall  $\times$  tall crosses produce dwarfs in  $F_2$  and subsequent generations, the genetic composition of the parents is *DDII* and *ddii*; (2) when tall  $\times$  tall crosses fail to produce dwarfs the parental types are both either *DDII* or *ddii* and, therefore, dwarfs are not to be expected. In addition to these, there appears to be at least one other type, and possibly more. This conclusion is based on a study of intercrossing varieties some of which were known from previous studies to be of a certain genetic composition in respect to the dwarfing character. In Tables 11 and 12 are shown all the crosses upon which the conclusions herein reported are based. In Table 11 are listed those crosses that gave rise to dwarfs in the progeny, and in Table 12 those that did not. Of the 92 crosses, 36 produced dwarfs in the progeny.

TABLE 11.—Crosses that gave rise to dwarfs in  $F_2$  and subsequent generations

Cross No.	Female parent	Male parent
A to G	Dicklow	Sevier.
1	do	Federation.
Q	do	Hard Federation.
23a	Sevier 121	Dicklow No. 4.
31	Marquis	Federation.
35	Dicklow No. 3	F-22 (Dicklow $\times$ Sevier).
36	do	Sevier No. 59.
39	Hard Federation	Dicklow No. 3.
40	Turkey	Federation.
47	Marquis	Hard Federation.
49	Hard Federation	Hussar.
50	Federation	Do.
53	Martin	IIIc-8 (Dicklow $\times$ Sevier).
56	G-149 (Dicklow $\times$ Sevier)	Martin.
57	Martin	G-149 (Dicklow $\times$ Sevier).
67	Ridit	Sevier No. 59.
68	do	15a-318 (Kanred $\times$ Sevier).
69	do	Utac (Dicklow $\times$ Sevier).
70	do	14-85 (Federation $\times$ Sevier).
72	do	Federation.
76	Hard Federation	Ridit.
103	Federation	Martin.
No number	Hard Federation	Hohenheimer.
105	do	Heil Dickkopf.
No number	Federation	Do.
107	do	Hohenheimer.
113	Ridit	15a-261 (Kanred $\times$ Sevier).
116	do	15a-890 (Kanred $\times$ Sevier).
117	do	16a-1429 (Odessa $\times$ Sevier).
118	do	16a-1607 (Odessa $\times$ Sevier 101).

TABLE 12.—Crosses that did not give rise to dwarfs in the progeny

Cross No.	Female parent	Male parent
4 to 14	Federation	Sevier.
15	Kanred	Do.
16	Odessa	Sevier No. 101.
17	do	Sevier No. 59.
18a	Federation	Do.
18b	do	Do.
18c	do	Sevier No. 88.
18d	do	Sevier No. 86.
18e	do	Sevier No. 100.
28	do	F-22 (Dicklow × Sevier).
29	G-149 (Dicklow × Sevier)	Federation.
30	Federation	IIIc-18 (Dicklow × Sevier).
32	Hard Federation	Kota.
33	Kota	Hard Federation.
34	Hard Federation	F-22 (Dicklow × Sevier).
38	G-149 (Dicklow × Sevier)	Hard Federation.
41	Odessa	Do.
42	do	Federation.
43	Hussar	Turkey.
51	Kanred	Hussar.
54	Ridit	Kanred.
59	Hybrid 128 × White Odessa	Do.
66	do	Sevier No. 59.
71	Ridit	Turkey No. 989.
73	Federation	Forward.
84	do	5-69-3 (Federation × Sevier).
87	Hope	Dicklow No. 3.
88	Preston C. I. 3081	Federation.
89	O1-24 <sup>a</sup>	14-85 (Federation × Sevier).
90	Dicklow No. 3	O1-24 <sup>a</sup> .
91	Hope	Federation.
92	Preston C. I. 3081	Dicklow No. 3.
93	do	O1-24 <sup>a</sup> .
94	Marquis	Dicklow No. 3.
100	Silvercoin	Heil Dickkopf.
101	Goldcoin	Hohenheimer.
102	Ridit	Silvercoin.
104	Goldcoin	Sevier No. 59.
106	Silvercoin	Do.
108	do	Kanred.
109	Hard Federation	Forward.
110	Forward	Hohenheimer.
111	do	Heil Dickkopf.
112	Ridit	15a-267 (Kanred × Sevier).
114	do	15a-400 (Kanred × Sevier).
115	do	15a-883 (Kanred × Sevier).

<sup>a</sup> Probably Dicklow × Federation, parents not known.A NEW GENETIC TYPE, *ddII*

In the tall × dwarf crosses it was established that Dicklow and Marquis are of the *ddii* type, while Federation, Hard Federation, and Sevier 59 are of the *DDII* type. Thus, the results of some of the following crosses are difficult to explain if only two types of tall exist:

Hope × Dicklow 3	No dwarfs in progeny.
Hope × Federation	Do.
Preston × Dicklow 3	Do.
Preston × Federation	Do.
O1-24 × Dicklow 3	Do.
O1-24 × Preston	Do.
Silvercoin × Heil Dickkopf	Do.
Goldcoin × Hohenheimer	Do.
Silvercoin × Sevier 59	Do.
Goldcoin × Sevier 59	Do.
Hard Federation × Forward	Do.
Hohenheimer × Forward	Do.
Federation × Hohenheimer	Dwarfs in progeny.
Federation × Heil Dickkopf	Do.

Since Dicklow 3 is of the *ddii* type, and when it was crossed with Hope no dwarfs appeared, Hope would be expected to be *ddii*. However, the fact that Federation is of the *DDII* type and when crossed with Hope gave no dwarfs proves that Hope is not of the *ddii* type. It is evident that Hope could not be *ddii* in one cross and *DDII* in another. However, if it is considered that Hope is of the new type *ddII*, then dwarfs are not expected in either cross since *ddii* × *ddII* and *DDII* × *ddII* would not throw dwarfs. This is due to the fact that in the first cross there is no dwarfing factor present and in the second cross the inhibiting factor, *II*, would always be present. For the same reason Preston would also be of the *ddII* type.

The fact that Dicklow 3 × 01-24 and 01-24 × 14-85 (Federation × Sevier) gave no dwarfs makes it appear logical to conclude that 01-24 is of the *ddII* type. Federation × Hohenheimer gave dwarfs; therefore, Hohenheimer is of the *ddii* type. However, both Goldcoin (Fortyfold) × Hohenheimer and Goldcoin × Sevier 59 gave no dwarfs; and, furthermore, since Sevier 59 is of the *DDII* type Goldcoin must be of the new type, *ddII*. By a similar analogy Silvercoin and Forward are of the new type, *ddII*. Thus it is apparent that there are at least three genetic types. A genetic classification of strains and varieties into the three types is given in Table 13. The *ddII* type is readily obtained when the other two types, *DDII* and *ddii*, are crossed.

TABLE 13.—Genetic classification of wheat varieties and strains used in the various crosses, based on their behavior to the dwarfing versus tall characters

DDII	ddii	ddII
Federation.....	Dicklow.....	Forward.
Hard Federation.....	Dicklow 3.....	Goldcoin.
Kota.....	Dicklow 4.....	Hope.
Odessa <sup>a</sup> .....	Heil Dickkopf.....	Kanred.
Sevier.....	Hohenheimer.....	Preston 3081.
Sevier 59.....	Hussar.....	Silvercoin.
Sevier 86 <sup>a</sup> .....	Marquis.....	01-24 (possibly Dicklow × Federation).
Sevier 88 <sup>a</sup> .....	Martin.....	
Sevier 100 <sup>a</sup> .....	Ridit.....	15a-267 (Kanred × Sevier).
Sevier 101 <sup>a</sup> .....	Turkey.....	15a-400 (Kanred × Sevier).
Utac (Dicklow × Sevier).....	Turkey 989 <sup>a</sup> .....	15a-883 (Kanred × Sevier).
III C-8 (Dicklow × Sevier).....		
III C-18 (Dicklow × Sevier) <sup>a</sup> .....		
F-22 (Dicklow × Sevier).....		
G-149 (Dicklow × Sevier).....		
5-69-3 (Federation × Sevier).....		
14-85 (Federation × Sevier).....		
15a-261 (Kanred × Sevier).....		
15a-318 (Kanred × Sevier).....		
15a-890 (Kanred × Sevier).....		
16a-1429 (Odessa × Sevier).....		
16a-1607 (Odessa × Sevier).....		
Hybrid 128 × White Odessa <sup>a</sup> .....		

<sup>a</sup> Some of these may be of the new type, *ddII*.

It will be seen from the classification of strains and varieties in Table 13 that strains 15a-267, 15a-400, and 15a-883 are of the new type (*ddII*) coming out of a Kanred (*ddII*) × Sevier (*DDII*) cross, whereas 15a-261 and 15a-890, out of the same cross, are *DDII*.

Strain 01-24 (possibly Dicklow × Federation) is of the new *ddII* type. If Dicklow and Federation are the true parents of 01-24 we have a new genetic type of tall (*ddII*) produced by crossing *ddii* (Dicklow) and *DDII* (Federation).



To determine further whether the proposed genetic composition of the new type is correct, crosses involving dwarfs and the new types were made. Theoretically, the  $F_2$  should give a 13:3 ratio and the  $F_3$  the usual 7:6:2:1 (7:4:2:2:1) ratio. However, the data from these crosses are not yet available.

It is not to be inferred from the data in Table 13 that this is a final classification. It is possible that some modification may be necessary as additional information becomes available. However, the genetic interpretation proposed provides a logical explanation of the data secured from a detailed study of the 15 crosses as well as from observations on 63 others, involving 50 strains and varieties of wheat.

### SUMMARY

The results of a genetic study of dwarfing in wheat secured from 15 crosses and from observations on the occurrence of dwarfing in 77 others are reported.

Dwarf plants are grasslike clumps and are rather variable, especially as to height, leafiness, and earliness of maturity. Dwarfing is the result of a distinct shortening of either the lower second or third internode or both. Studies in the  $F_2$  and  $F_3$  generations in four spring-wheat crosses gave results which were explained on a 2-factor basis; that is, a dominant dwarfing factor, *DD*, and an inhibiting factor, *II*.

From a study of the  $F_3$  breeding behavior of the tall  $F_2$  plants from seven winter-wheat crosses, it was concluded that the dwarfing character could be explained on the usual 2-factor basis.

Results secured from four tall  $\times$  dwarf crosses gave further proof of the 2-factor theory for dwarfing and also established the genetic composition of a number of varieties in regard to this character.

One tall  $\times$  dwarf cross gave irregular breeding behavior in  $F_3$ . The ratio expected was 1 (tall) : 2 segregating : 1 dwarf. It happened that there were too many in the segregating class and too few in the true-breeding dwarf class. These results were explained on the assumption that natural crossing of tall and dwarf plants had occurred in the  $F_2$  generation. Data are presented showing that natural crossing of tall and dwarf plants may be of common occurrence.

A biometrical study of the culm length of  $F_2$  genotypes, based on the  $F_3$  breeding behavior, is presented. The dwarfs in two of the crosses were distinctly outside the range of tall plants. In the other crosses there was considerable overlapping. In length of culm dwarf plants were considerably more variable than tall plants. Tall plants, in spring-wheat crosses grown under irrigation, showed slightly more variation in length of culm than did the tall plants in winter-wheat crosses grown under dry-land conditions.

Intercrossing varieties, some of known genetic composition, gave conclusive evidence that among the tall plants there are more than those of the *DDII* and *ddii* types. The new type is *ddII*. It is readily produced by crossing the other two types (*DDII* and *ddii*). With this additional type it was possible to explain the breeding behavior observed in all the intervarietal crosses studied.

A tentative genetic classification in respect to dwarfing is presented for the varieties and strains used in the crosses.

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## COMPARATIVE SEROLOGICAL AND PATHOLOGICAL INVESTIGATIONS OF THE FIRE-BLIGHT ORGANISM AND A PATHOGENIC, FLUORESCENT GROUP OF BACTERIA<sup>1</sup>

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### INTRODUCTION

One of the most vexing problems in bacteriological research is the identification of the bacterium under investigation. As long as writers continue to describe new species on the basis of a few unverified or even verified cultural, physiological, or morphological differences within a given group of bacteria, the problem is likely to remain complex and almost insolvable. On such a basis of identification and classification one is justified in considering almost every isolate in certain groups of bacteria as representing a distinct species.

The value of serological investigations as an aid in identifying and classifying various human and animal pathogens has been recognized for about 40 years as a highly important and almost indispensable procedure. Within recent years plant pathologists have likewise utilized such tests to some extent and in a few instances have conclusively shown (21, 22, 26, 27, 28, 29)<sup>2</sup> that important clues to the identity and relationship of various perplexing plant pathogens can be furnished by serological investigations. In view of the extensive series of consistent results here reported, it is hoped that, aside from the information obtained concerning the bacteria under investigation, these will offer additional evidence, if any more is needed, of the value of such studies in determining relationships among bacterial plant pathogens.

For the last six years the senior writer has been studying the highly destructive disease of pears and apples known as fire blight. Part of these studies consisted of the gathering of empirical field data relative to the prevalence and destructiveness of the disease, varietal and species behavior of hosts, the observance of overwintered cankers and blighted organs of various types, the relationship of such blighted tree parts to the location of new infections, the relationship of insect carriers and rain and wind to disease dissemination, and observance of the organs first to show signs of the disease in the early spring. During the first three years of these investigations it was assumed that when a flower, leaf, twig, or limb of the proper host showed signs of blighting and the presence of bacteria within the tissues, fire blight was present. In many instances this was abundantly confirmed by pure culture isolations, type of growth reactions of the isolates<sup>3</sup> on various culture media, chemical reactions, pathogenicity tests, and

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 117.

<sup>3</sup> The very convenient term "isolate" recently proposed by Brierley (7), is adopted because it avoids the use of the word "strain" which is often used indiscriminately to designate a genotypic or phenotypic variant, and also to designate a particular organism isolated at a given time without reference to its taxonomic position. "Isolate" is applied specifically to the latter.

morphological studies. Over 200 of such isolates were studied and the external and internal symptoms of the natural infections from which they were derived amply observed, as were the artificial infections induced by them. Yet no suspicion arose until two years ago that the maladies considered as one disease may possibly comprise two distinct diseases induced by two different species of bacteria.

#### DISCOVERY OF TWO DIFFERENT BACTERIAL DISEASES OF POMES IN ARKANSAS

The possibility that two bacterial diseases occur on pears and apples first presented itself in a study of the blighting of unopened pear and apple blossoms. When such blighted blossoms were found during the first few years of these investigations, the disease was attributed to frost or insect injury or to the fire-blight pathogene, and so recorded (34). Not until the data for several years had accumulated relative to the absence of infectious exudate at the time of the first spring blossoms (35, 36) and not until it was observed that with rare exceptions fire blight appeared first on fully opened blossoms, many of which had already been pollinated and had set fruits, did it seem worth while to study thoroughly those exceptional cases in which closed blossoms of early season formation were blighted and in which bacteria, presumably *Erwinia amylovora*,<sup>4</sup> were present. When several pure culture isolations from closed blossoms were carefully investigated and compared with *Erwinia amylovora* as to cultural reactions, chemical tests, morphological details, and pathogenicity, it was discovered that, with the exception of certain marked differences in symptoms in artificial inoculations, pigment production on beef-infusion agar and synthetic media, there were only slight differences or none at all in by far the largest number of tests. The chemical tests included those for the production of amylase, invertase, pectinase, ammonia, nitrite, indol, hydrogen sulphide, the digestion of milk and gelatin, and acid and alkali production. Obviously, unless one were prepared to undertake an extensive series of trials on numerous culture media, any dissimilar bacterium isolated would very likely be overlooked and would be grouped with *Erwinia amylovora*.

After it was once found that bacteria isolated from early spring blossom infections on pears produced a greenish-yellow or bluish-yellow fluorescent pigment on certain nutrient media, that their period of incubation is shorter than that of *Erwinia amylovora*, and that they produce an intense blackish discoloration of tissues along veins, midribs, and laminar tissue between veins (fig. 1) considerably ahead of bacterial penetration, it was not difficult to find numerous comparable bacteria in the early part of the growing season. The four isolates later referred to as Petal, Receptacle, Lincoln, and Garber are typical of this species and represent the kind of bacteria often present in closed, blighted blossoms of the early season, the kind present in early season leaf spotting, and in the blighting of young pear fruit.

The disease, which for convenience is now designated pear and apple blast, caused by these early season isolates in contrast to that caused by *Erwinia amylovora*, is typically a cool-weather malady. As soon as warm weather sets in it is found much less frequently. In this respect, the disease is quite comparable to citrus blast and

<sup>4</sup> *Erwinia amylovora* and *Bacillus amylovorus* are used synonymously in this paper.

black pit, diseases caused by a bacterium very closely related to, if not identical with, that isolated from diseased pomes, and the temperature relationships that have been found to exist (43) between the citrus-blast organism and the size of spots on lemon fruits suggest a similar relationship on pomaceous hosts.

Are the differences previously enumerated sufficient to indicate that the pear and apple blast disease is distinct from fire blight and that it is caused by a different pathogene? How may the numerous points of similarity be explained? In an effort to answer these questions the serological investigations reported in the present paper were undertaken.

Baumgärtel (4) has found that—

As is evident from precipitation and agglutination reactions, different kinds of bacteria grown on the same nutrient medium and under conditions precisely

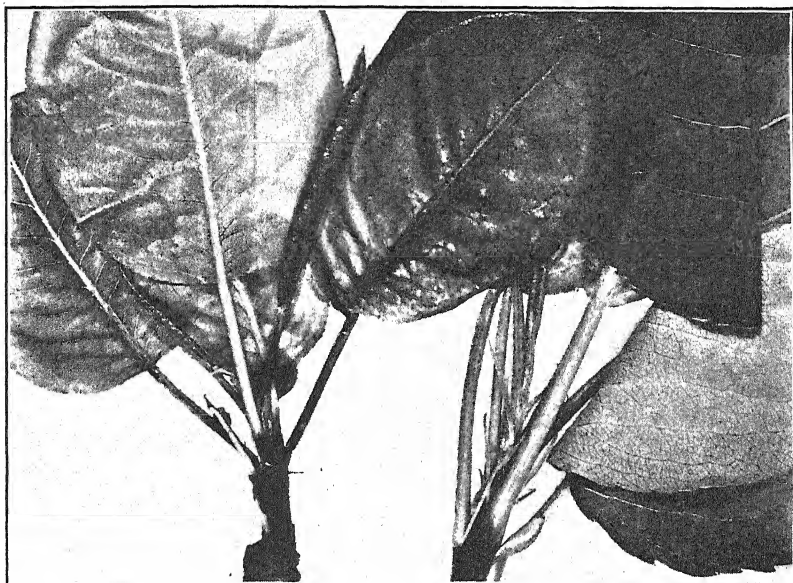


FIGURE 1.—Artificial inoculations on Bartlett pear with two of the pear-blast isolates used in the serological tests. Receptacle, right; petal, left. Photographed 20 hours after inoculation. Note the blackening of the tissues along the veins of the leaf near the center, a typical symptom of pear blast in artificial infections

similar in other respects can be differentiated with respect to their proteins, so that with the aid of the serological reactions they can be identified without further difficulty. Chemical analysis, on the other hand, offers at present no method of distinguishing the different kinds of bacterial proteins, for the methods are not sensitive enough and do not give unconditionally reliable results; nor do they permit generalization.

After it was determined that numerous isolates from pear blast (apple-blast isolates have not been so thoroughly studied up to the present) all produce a fluorescent pigment, as previously noted, a number of other fluorescent bacteria described previously as pathogenic to rosaceous plants were included in the serological tests, as well as several isolates of typical *Erwinia amylovora*. The following fluorescent species were considered: *Phytomonas syringae* (Van Hall) Bergey et al., *P. cerasi* (Griffin) Bergey et al., *P. citriputeale* (C. O.

Smith) Bergey et al., *P. papulans* (Rose) Bergey et al., *P. nectarophila* (Doidge) Bergey et al., *P. barkeri* Bergey et al., *P. prunicola* (Wormald) Wormald, and *P. utiformica* Clara.<sup>5</sup> A brief résumé of

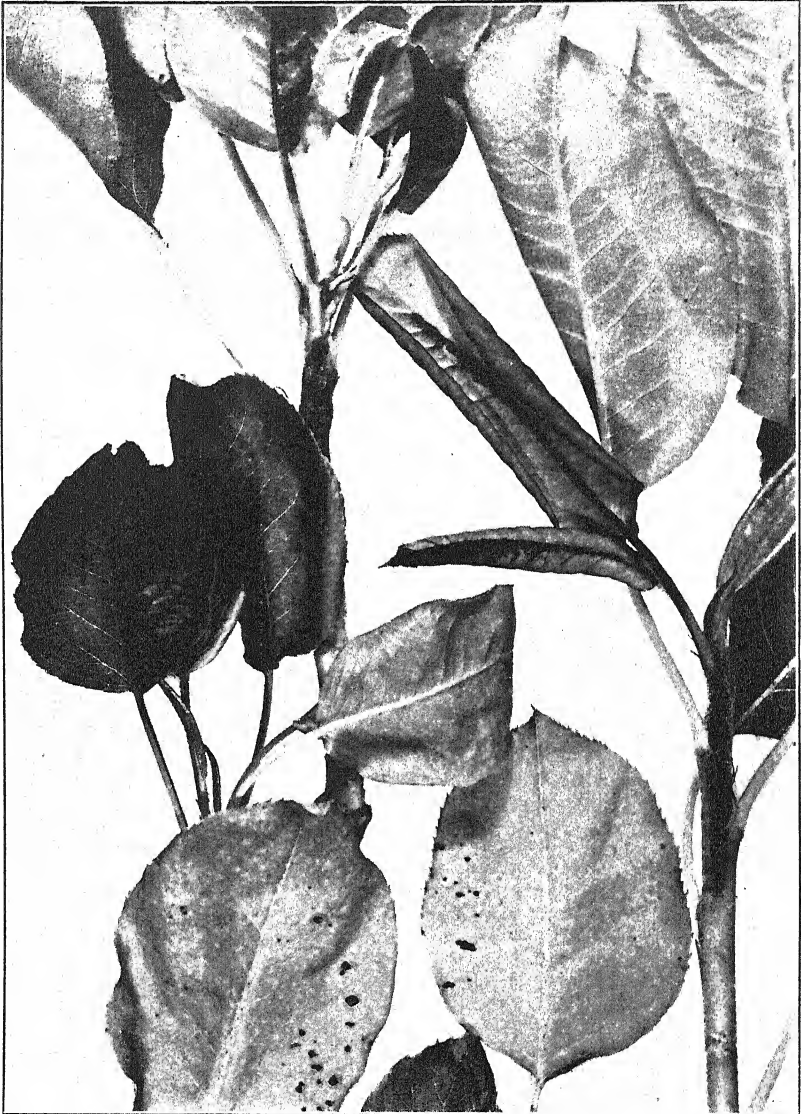


FIGURE 2.—Artificial inoculations on Bartlett pear with pear-blast isolate, Receptacle (upper shoot), and with *Phytophthora syringae* on the remainder. The two middle shoots were inoculated by means of a hypodermic needle and the lower leaves by means of a spray with a bacterial suspension in sterile water on uninjured leaves. Compare with Figures 1 and 5

these fluorescent bacteria, a list of assigned host plants, and tests involving the present writers' pathogenicity studies made with some of these organisms follow.

<sup>5</sup> Clara calls this *Pseudomonas utiformica* sp. nov.

Cultures of *Phytomonas syringae* and *P. citriputeale* were obtained from Mary K. Bryan and compared with pear-blast isolates in morphology, cultural and physiological tests, and in pathogenicity. It was found that, while there are some differences, the organisms as a whole are a rather uniform group and that the differences between them are no greater than those between various pear-blast isolates. The symptoms produced on pear and apple by the lilac-blight organism are hardly to be distinguished from those brought about by the pear-blast isolates. (Fig. 2.) Likewise the symptoms on oranges and lemons produced by the pear-blast organism and the lilac-blight organism are directly comparable with those that have been found for the citrus blast organism. The culture of *P. citriputeale* available to the writers apparently had lost most of its virulence, as it produced no noticeable infections on orange or lemon fruit; and although 30 hypodermic injections were made into succulent Bartlett-pear shoots, only three shoots showed small more or less localized lesions as a result. The culture produced no infections on unwounded pear leaves or twigs in contrast with pear-blast isolates and the lilac-blight organism (fig. 2) which readily produced infections on unwounded tissues. From one of the lesions produced on pear by *P. citriputeale* the organism was reisolated, and when it was again inoculated into pear it caused somewhat larger spots to appear.

Wormald furnished a culture of his organism, which he named *Phytomonas prunicola*, and for the last year or more, December, 1930, to February, 1932, it has been carefully studied simultaneously with various pear-blast isolates, as well as with *P. syringae* and *P. citriputeale* (*P. cerasi* was unavailable). A very characteristic reaction of all of these is the production of a greenish-yellow, bluish-yellow, or bluish-green fluorescent pigment on beef-infusion agar and on various synthetic media, including Uschinsky's solution. The amount and quality of pigment varies somewhat with the different isolates and even with the same isolate at different times, and what one individual would class as green pigment may very readily be classed as yellow by another. As a matter of fact, the fluorescent pigment may be visualized as predominantly blue, green, or yellow, the color, aside from any true difference in strains or isolates and from the factor commonly known as the personal equation, depending on the quality and quantity of light in which the cultures are observed. Thus, while Wormald describes the pigment of his organism as yellow in Uschinsky's solution, the senior writer would call it opalescent or fluorescent greenish blue in transmitted light, and when culture tubes are held alongside of Ridgway's color charts,<sup>6</sup> a diffuse, reflected northern light being used, the color falls between Chrysolite green and Kildare green. The color also varies with the age of the culture. It would appear, therefore, that on the basis of pigment production it would be impractical to separate *P. prunicola* from *P. cerasi* and from the other organisms named above. As will be more fully discussed later, *P. prunicola* can hardly be distinguished from *P. syringae* and from the pear-blast pathogene either in pathogenicity tests (figs. 3 and 4) or in cultural reactions, and the serological tests further emphasize its relationship to this group.

<sup>6</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.



## PREVIOUS INVESTIGATIONS ON THE FLUORESCENT BACTERIA

The lilac-blight organism, *Phytomonas syringae*, first described by Van Hall in 1902 (19) and more recently by Bryan (9), is not only pathogenic to lilac but also to pear and apple, cherry (*Prunus mahaleb*), poplar (*Populus nigra*), buckwheat (*Fagopyrum esculentum*), and



FIGURE 3.—Artificial inoculations with *Phytomonas prunicola*, the English plum-wilt organism, on Bartlett-pear shoots. Compare with Figure 1

orach (*Atriplex hortensis*), as Van Hall determined in artificial inoculations. In addition to these hosts it has been found by Smith (40, 41) to attack citrus, producing symptoms that are indistinguishable from those produced by the citrus blast and black-pit organism, *P. citriputeale*. In cultural characteristics the latter organism showed "a great similarity" to the lilac-blight organism. Smith likewise found a similar relationship with the pathogene concerned in gummosis and canker of stone fruits, *P. cerasi* (41).



With each of these three organisms, *P. syringae*, *P. citriputeale*, and *P. cerasi*, he produced artificial infections on *Prunus armeniaca*, avocado, *Chalcas exotica*, *Coprosma baueri*, *Jasminum primulinum*,

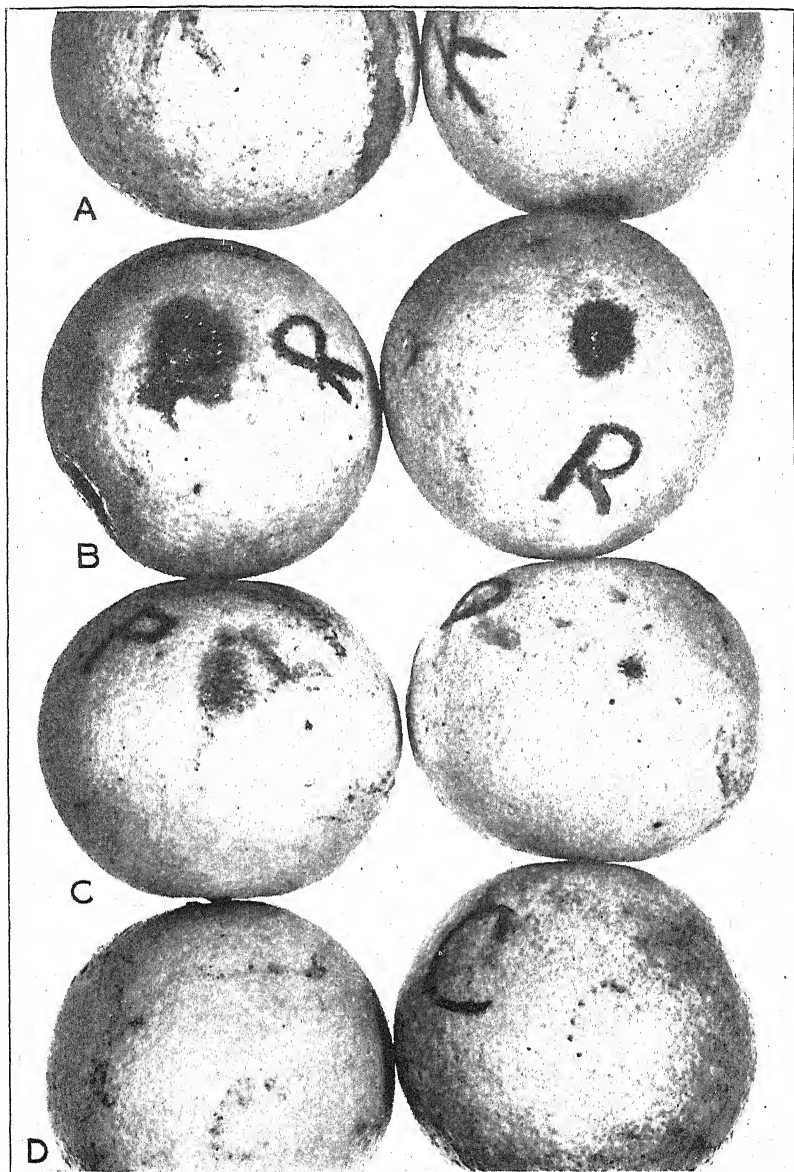


FIGURE 4.—Artificial inoculations on orange, by means of needle pricks, with pure cultures of *Erwinia amylovora* (A), pear-blast isolate, Receptacle (B), *Phytophthora prunicola* (C), and checks, sterile water (D). Photographed six days after inoculation. Note absence of infection with *Er. amylovora* and in checks, and the typical black-pit infections, characteristic of citrus blast, with pear-blast isolate and with *P. prunicola*.

*Fraxinus floribunda*, lemons, oranges, tomato fruit, lilac, *Populus* sp., pears, and apples. Bryan (9) confirmed Smith's findings con-

cerning the similarity of *P. syringae* and *P. citriputeale* and decided that the two are indistinguishable. Smith, however, jointly with Fawcett (43, p. 245), concluded that "with our present knowledge of this group the authors would hesitate to place these three organisms \* \* \* in a single species."

A fluorescent bacterium was described in 1917 by Rose (31) as the cause of a blister spot on apple fruits. He named it *Pseudomonas papulans* and presented some evidence indicating that a scurfy bark disease on apples may be due to the same or closely related organisms. In 1924, Rhoads (30), describing the disease known as apple measles and including under this a scurfy-bark type, concluded that it does not appear to be caused by any microorganism but is a nonparasitic (physiologic) disturbance. Hopkins<sup>7</sup> working in South Africa reported in 1927 a bark disease of apples consisting of blisterlike lesions which make their first appearance at the bases of the trees, whence they spread upwards and girdle the trunks. He also found the disease on young twigs and noted the similarity of bark symptoms to fire blight and to Rose's scurfy-bark disease. Several isolations from the spongy layer under the loosened epidermis gave "practically pure cultures of a bacterial organism," and comparable bacteria were isolated from other portions of the lesions. He succeeded in producing typical infections with these and in recovering the organism from the artificial inoculations. Some of the isolates produced a fluorescent pigment on certain media, while others were free from this, though he evidently decided that all the isolates represented but one species and that it was distinct from *Erwinia amylovora* and *Phytophthora papulans*.<sup>8</sup> Lacey and Dowson (20, p. 36) in 1931 described a bacterial canker of apple trees in England and concluded that the causal organism which produced a fluorescent pigment on some media "bears a certain likeness to *B. amylovorus*, but it is regarded as identical with *Ps. papulans* Rose."

In view of the doubt thrown upon the etiologic relationship of *Phytophthora papulans* by Rhoads and others, it may be desirable to record briefly that several isolations made by the senior author in October, 1924, from blackish depressed spots on Stayman Winesap apples gathered at Gravette, Ark., yielded cultures of a fluorescent bacterium that produced comparable symptoms on apple fruit when inoculated by means of needle pricks. These isolates have since been lost and will not be further considered.

A bacterial disease of pear blossoms in England was first briefly described in 1913 by Barker (1), and amplified by Barker and Grove (2) in 1914, who recorded, in addition to an attack on pear blossoms, a leaf spotting, and a dieback of spurs. Also, "there is reason to believe that a number of other plants are susceptible," including flowers of apples, cherries, gooseberries, and plums. They further concluded that the causal organism is disseminated by bees and other insects, as they obtained the typical species of bacteria in 50 per cent of the cases when a number of bees that had been caught while working on pear blossoms were introduced on culture media in Petri dishes. They also claimed to have found the organism in the soil of fruit plantations. Cultural and morphological characters were

<sup>7</sup> HOPKINS, C. J. AN APPLE DISEASE OCCURRING IN THE ELGIN DISTRICT. Union So. Africa Dept. Agr. Sci. Bul. 61, 17 p., illus. 1927. [Mimeographed.]

<sup>8</sup> Hopkins uses the terms "*Bacillus amylovorus*" and "*Pseudomonas papulans*."

presented, including the production of a greenish fluorescence on glucose-peptone media. The organism, however, was not named until 1924, when Berridge (6) called it *Bacillus barkeri*.

While the senior writer has been unsuccessful in producing infections with *Phytomonas barkeri* on either pear flowers or succulent twigs or leaves with two different cultures, one supplied by Barker and the other by St. John-Brooks, curator of the national collection of type cultures (Great Britain), nevertheless, there is good reason for believing that at least some of the isolates investigated by Barker and Grove represented a pathogenic species. Doidge (12) succeeded in producing black spots on pear receptacles with the culture that Barker and Grove sent. Wormald's (47, p. 131-132) brief report of a bacterial blossom wilt of pears and a bacterial fruit rot of cherries in England, with symptoms "similar to those of a disease which has been studied at the Long Ashton Research Station," indicates that a bacterial disease of pear blossoms is present in England and that it is produced by a fluorescent pigment bacterium, since Wormald reports that the isolates are closely related to his *Pseudomonas prunicola*.

In 1917 Doidge (12) described a bacterial blight of pears in South Africa and named the causal agent *Bacterium nectarophilum*. A comparative study of Barker and Grove's organism and the South African one was made with reference to cultural and physiological reactions and morphology. At the same time the published accounts of *Erwinia amylovora* were used as a further guide. The main differences noted between the South African organism and the English one were the presence of capsules in the former, none in the latter (Smith and Fawcett (43) report that citrus, Prunus, avocado, and lilac isolates are all encapsulated), the optimum temperature for growth, given as 25° to 30° C. for the South African organism and 15° to 18° in the English, and absence of gelatin liquefaction in the first and its presence in the second. (When the citrus-blast organism was grown continuously on potato-glucose agar for a year or more, Smith and Fawcett (43) found that its ability to liquefy gelatin is decreased or is absent.) However, the rods are comparable in size, both are polar flagellated, both produce a fluorescent pigment, though this is more noticeable in the South African pathogene, and both present similar cultural and physiological reactions on a relatively wide range of culture media. The two isolates of *Phytomonas barkeri* which the senior writer studied and which were found to be nonpathogenic under greenhouse conditions of relatively high temperatures, nevertheless grew very well at incubating temperatures of 25° to 30° C. on various culture media. As the writers have been unable to procure cultures of *Bacterium nectarophilum* from Miss Doidge, it is somewhat hazardous to make any positive statement relative to the relationship of the South African pear blossom-blight producer to other described pear pathogenes, but the fact that it belongs to the fluorescent group and possesses numerous other features characteristic of the bacterial pathogenes typified by the pear-blast organism, suggests that for the present it would be preferable to consider it a closely related variety or strain.

In 1930 Wormald (46) described a bacterial disease of plums involving a wilting of shoots and a spotting of leaves in natural infections. In addition to these, he also obtained a killing of buds and a

spotting of fruit in artificial infections with pure cultures. He studied the cultural characters of the pathogene carefully and compared them with the published descriptions of *Erwinia amylovora*, *Phytomonas pruni*, *P. cerasi*, and *Pseudomonas*<sup>9</sup> *spongiosa*. He found the group number of *Er. amylovora* as given by Stewart (44) to be exactly the same as that for the English plum-wilt organism, and the chief difference noted between the two was in the arrangement of flagella, that in the fire-blight pathogene being considered peritrichic and in the plum-wilt organism polar. As *P. pruni* is a yellow organism it is excluded from further consideration, and Wormald's exclusion of Aderhold and Ruhland's *Pseudomonas spongiosa* is doubtless justified because of their very brief and inadequate description. But his exclusion of *P. cerasi* because it "is stated to be a green organism" is not in accord with Griffin's (15) original description which reads, "\* \* \* A chromogenic feature appears when my organism is grown on certain media, namely, a greening of the agar in plates, stabs, and slants \* \* \*."

After the writers had completed their studies on serological reactions of *Phytomonas prunicola*, *Erwinia amylovora*, and pear-blast pathogene and had concluded that *P. prunicola* was not to be distinguished from the latter, Smith (42) very briefly announced that *P. prunicola*, a culture of which he had obtained from Wormald, was "apparently very similar to, if not identical with, \* \* \* *Bact. syringae*, \* \* \* with *Bact. citriputale*, and with \* \* \* *Bact. cerasi* \* \* \*." Likewise, Wilson (45) in a comparison between *P. prunicola* and two different types of isolates from gummosis lesions of plum and apricot trees in California concluded that Wormald's organism resembles very closely one of his California isolates.

Shortly after the senior writer's (37) preliminary announcement of a new pear and apple disease in North America, simulating fire blight, and having as its etiologic agent a pathogene belonging to the group typified by *Phytomonas barkeri*, *P. nectarophila*, and *P. prunicola*, Clara (11) published a brief account of a new pear disease (from New York State) caused by a greenish, fluorescent bacterium. While noting that it is closely related to *P. syringae* and other fluorescent pathogenes, he nevertheless decided that it was not identical with these and was a new species, *Pseudomonas utiformica*. "\* \* \* a comparison with the descriptions of the known species of these bacterial plant pathogenes found on pear, *Pseudomonas barkeri* and *Pseudomonas nectarophila*, showed no identity."

Despite the fact that 30 years ago Van Hall (19) had found that *Phytomonas syringae* is capable of attacking a number of unrelated plants including members of the Salicaceae, Polygonaceae, Chenopodiaceae, and Rosaceae, in addition to lilac (Oleaceae), his work has largely been disregarded, and species making of fluorescent pathogenes has continued unabated. This is in part explained by the fact that aside from lilac which was found naturally infected, the other hosts were only infected artificially by Van Hall. In other groups of microorganisms, such as the rusts, smuts, powdery mildews, and downy mildews, artificial infections on different hosts have become widely recognized as offering valuable criteria in determining

<sup>9</sup> To avoid making a new combination of a somewhat doubtful species the generic name *Pseudomonas* is retained, otherwise it belongs in *Phytomonas*.

the identity of any given fungus. Indeed such work holds exceptional interest because field behavior can be predicted by means of artificially induced infections.

Rosen and Groves (38), using pure cultures of *Erwinia amylovora*, were able to produce abundant infections by means of artificial inoculations on *Spiraea vanhouttei*, *Chaenomeles lagenaria* (Japanese quince), and on cultivated rose. Shortly after these results were published natural infections on *S. vanhouttei* were found in Virginia (16), and private reports from California and Missouri indicate that such infections were found on Japanese quince and on rose.

Among the first to investigate bacterial phytopathogenes by serological methods were St. John-Brooks, Nain, and Rhodes (39). They included in their studies some 13 named pathogenic species of fluorescent bacteria, including *Phytomonas barkeri* and *P. syringae*, as well as 4 nonpathogenic isolates of *Pseudomonas fluorescens* for comparison with pathogenic species. Unfortunately they made no tests for pathogenicity, so that the question may be raised as to the identity of at least some of their organisms. This is especially applicable to *P. syringae*, which in their experiments produced acid and gas in several different carbohydrate media, although several investigators, including Bryan (9), Smith and Fawcett (43), and the present writers, have noted no gas production for this species on any carbohydrate media. The production of gas by bacterial plant pathogenes is uncommon on such media. Of 93 species of such bacteria listed in Bergey's Manual (5), only 8 are noted as producing gas. This figure undoubtedly would be modified by closer study of the literature, but it is clearly indicative of the scarcity of gas producers among plant pathogenes. It is therefore to be questioned whether St. John-Brooks and his associates had pure cultures of *P. syringae*.

Using 16 different types of sera, including *Phytomonas syringae* and *P. barkeri* against 18 antigens, including the 2 just mentioned, at a dilution of 1 to 100, St. John-Brooks and his associates obtained with *P. syringae* serum agglutination only against the homologous antigen and against *P. atrofaciens*, while with *P. barkeri* serum agglutination was noted in the homologous antigen and in *P. tolaasi*. In view of the question raised above relative to the identity of *P. syringae* among St. John-Brooks, Nain, and Rhodes's cultures and in view of the low dilutions attempted, higher ones being contemplated for the future, one may feel somewhat doubtful as to the significance of their work on the two organisms noted, although their pioneer efforts as a whole have been extremely valuable in calling attention to the importance of serological investigations of plant pathogenes.

Goldsworthy (14) is the only other investigator known to the writers who has attempted serological investigation of any of the fluorescent bacteria previously listed. Having experienced difficulty in distinguishing two different bacterial organisms concerned in the gummosis disease of stone fruit trees, *Phytomonas cerasi*, one of which produced a fluorescent pigment and the other did not, he decided to find out if agglutination tests would show the relationship between the two. Like other pigment producers, this one failed at times to produce pigment, so that the investigator could not tell which of the two organisms was being used at a given time. Goldsworthy found that the two would not cross agglutinate, and with homologous sera he obtained in three cases titers as high as 5,120. Had he used a larger

number of isolates, it is quite probable that he would have obtained entirely different results in the cross-agglutination tests.

#### METHODS USED IN THE SEROLOGICAL INVESTIGATIONS

While the methods used in agglutination and precipitation tests are becoming more and more standardized, there are sufficient variations to make it desirable to set forth the exact procedure followed. Only important points will be enumerated.

In a preliminary test a heavy bacterial suspension in 0.85 per cent saline solution of one isolate of *Erwinia amylovora*, and a similar suspension of a pear-blast isolate, 1 c c of each were injected intraperitoneally into two rabbits in order to ascertain the effect of injecting live organisms. No ill effects were noted.

To determine the agglutinating property of the normal sera of the rabbits to be used, the animals, 14 in the first series and 16 in the second, were bled from the inferior auricular vein, the blood was allowed to clot, kept in an ice chest overnight, the clot cut from the tubes and centrifuged. The supernatant sera were then added to uniform bacterial suspensions in saline solution containing 0.5 per cent phenol, in quantities to give serum dilutions ranging from 1 to 10 up to 1 to 5,120. The type of antigen used against a given serum corresponded to the one which was to be used later in immunizing the particular rabbit. All the serological tubes were then incubated overnight at 37° C. and the readings made macroscopically the following day. No agglutinations were noted in the first series. In the second series rabbits Nos. 22, 23 (Garber antigen), and 24 (*Phytomonas syringae* antigen) showed agglutination in dilutions of 1 to 10 and 1 to 20. The remainder showed no agglutination.

The density of the antigens was standardized both in the injections and in the agglutination tests by comparing the suspensions in serological tubes with a properly diluted commercial antigen of *Brucella abortus*, which had given excellent results in previous agglutination studies. The standard used in the final agglutination tests approximated the turbidity of a tube containing about 2,000,000 bacteria per cubic centimeter, while the suspensions used for injection were about fifty times as heavy.

Two rabbits were used for each isolate, and separate agglutination tests were run for each.

The culture media on which the bacteria to be injected were grown consisted of 48-hour-old slants of a commercial dehydrated potato-dextrose agar, testing pH 5.75, and to avoid the possibility of antibody reaction against the protein of potato the writers followed Link and Link's (21) procedure of using beef-extract dextrose-agar slants for growth of the bacteria to be used in the final agglutination tests. The latter medium was also a commercial dehydrated product, testing pH 7.2, on which the bacteria were grown for two to four days, varying somewhat with the different isolates.

Five intraperitoneal injections were made into each rabbit, the first as a 1 c c suspension and the others, three days apart, as a 2 c c bacterial suspension in each injection.

Ten days after the final injection the blood was drawn from the auricular vein of each animal. It was allowed to remain in the ice box overnight, otherwise treated as the normal blood used as the

control, and the antiserum was treated in the same manner as the normal serum.

Sufficient blood was drawn in each case to permit reciprocal tests throughout, a procedure which, as will be later shown, is very important owing to the possibility of error in some tubes, in manipulating more than 1,100 tubes in rapid order. By reciprocal tests is meant not the use of heterologous antigen against a particular antiserum, although this was of course included in the tests, but the use of a given organism as an antigen and also as an antiserum against all the other organisms included in the tests. By this method any error in manipulation is very likely to be detected. Thus, the results of the agglutination tests with Lincoln antiserum and *Phytomonas prunicola* antigen, which are included in Table 1, are to be questioned since the reciprocal with *P. prunicola* as the antiserum and Lincoln as the antigen shows entirely different results, the latter being directly in harmony with all cultural and physiological reactions of the two, as well as being in harmony with the very similar isolate, Receptacle, which shows no such discrepancy.

After any antigen was brought to its required density of suspension, it was filtered through clean glass wool in order to remove any clumps or particles of media, and in a few instances where the resulting suspension was not entirely cleared of clumps by filtration, the suspension was centrifuged and the supernatant suspension alone used in the agglutination tests.

A control tube of phenolized, saline antigen without any serum added was used in each rack and compared throughout with the tubes containing the same antigen plus antiserum. No agglutination appeared in any of the controls.

To make sure that contamination had not occurred in any of the organisms that were to be used in the serological work, and also to determine their pathogenicity at a given time, every isolate was inoculated shortly before each serological test, by means of a hypodermic needle, into three succulent pear shoots attached to growing Bartlett pear trees maintained in a greenhouse. Suitable controls in the form of hypodermic injections with sterile water were included in every trial. Every organism used in the first series was thus found to be virulent and apparently free from contaminations.

#### ORGANISMS USED IN FIRST SERIES OF AGGLUTINATION TESTS

As previously noted, the agglutination tests were made to determine the relationship between the fire-blight pathogene, *Erwinia amylovora*, and the pear and apple blast pathogene on the one hand, and the relationship of the pear-blast isolates to other pathogenic, fluorescent organisms on the other.

The first series included 7 different isolates, 4 typifying the non-fluorescent *Erwinia amylovora* and 3 the fluorescent pathogenes. The 4 *Er. amylovora* isolates included (1) Hlg 12/17/30, representing an isolate from honeycomb material gathered December 17, 1930; (2) Amelanchier, representing an isolate from a blighted blossom cluster of a cultivated species of Amelanchier gathered May 7, 1929; (3) Kieffer leaf spot, representing an isolate from a blighted Kieffer pear leaf gathered in June, 1931; and (4) 728, representing an isolate from a Jonathan apple twig gathered March 21, 1928. The three



fluorescent organisms included (1) Receptacle, representing an isolate from a receptacle of a Kieffer pear blossom gathered March 26, 1930; (2) Lincoln, representing an isolate from a blackened calyx cup of a young Lincoln pear fruit gathered on April 20, 1931; and (3) *Phytomonas prunicola*, representing an isolate obtained from Wornald, which he found to be the cause of a blighting of plum shoots in England. All the fire-blight and pear-blast isolates used in this series as well as in the next represent material gathered in Arkansas.

The results of the first series of serological tests are presented in Table 1. There is necessarily a great reduction in exact details, particularly in the amount of agglutination obtained in the various dilutions and in the range of dilutions utilized for each antigen antiserum.

TABLE 1.—*Titers of the first series of agglutination tests*

Type of antigen	Titer of antisera indicated						
	Hlg. 12/17/30	Amelan- chier	Kieffer leaf spot	728	Recepta- cle	Lincoln	<i>Phyto- monas prunicola</i>
Hlg. 12/17/30	1-1, 280 1-5, 120 1-3, 200	1-1, 280 1-3, 200 1-1, 600	1-5, 120 1-5, 120 1-5, 120	1-2, 560 1-800 1-5, 120	1-20 1-80 1-40	1-80 1-40 0	1-20 1-10 1-20
Amelanchier	1-5, 120 1-3, 200 1-1, 600	1-5, 120 1-640 1-800	1-5, 120 1-5, 120 1-5, 120	1-5, 120 1-2, 560 1-1, 280	0 0 0	0 1-40 0	1-10 1-20 0
Kieffer leaf spot	1-1, 600 1-5, 120	1-2, 560 1-1, 600	1-1, 600 1-1, 280	1-1, 600 1-2, 560	0 0	0 0	1-80 0
728	1-10 0	0 0	0 0	1-40 1-20	1-160 1-1, 280	1-1, 600 1-2, 560	1-1, 280 1-1, 280
Receptacle	1-40 1-40	1-40 0	0 0	1-20 1-20	1-80 1-800	1-80 1-800	1-800 1-640
Lincoln	0 0	0 0	0 0	1-10 0	1-80 1-320	1-80 1-10	1-640 1-640
<i>P. prunicola</i>	0	0	0	0			

<sup>a</sup> The test with *P. prunicola* as antigen and Lincoln as antiserum is probably an error in final manipulation; see the reciprocal, with *P. prunicola* as antiserum and Lincoln as antigen.

Table 1 shows that the 4 isolates of *Erwinia amylovora* behave remarkably alike with respect to agglutination tests, and that the 3 isolates of fluorescent bacteria, 1 *Phytomonas prunicola*, the English plum-blight organism, and 2 isolates of American pear-blast producers, are also a homogeneous group. The 4 *Erwinia amylovora* isolates all show cross agglutination, the titers of the heterologous ones being of the same order as the homologous sera antigens. The table also shows that the *Erwinia amylovora* group present higher titers as a whole than does the fluorescent group. The same striking difference will also be noted in the later series.

It appears that, as far as these serological tests are concerned, the *Erwinia amylovora* group represents a separate and distinct species from the fluorescent group, there being very little or no cross agglutination between the two groups. Of the 48 groups of cross-agglutination tests 25 showed no reaction whatever while the remainder showed titers of such low order as to be of little significance. Serological workers in general are in agreement that titers as low as 1 to 160 are of doubtful value, being considered either as nonspecific group reactions or as indicating some process unrelated to the production of specific agglutinins.



All the tests, with the exception of the results shown in Lincoln antiserum and *Phytomonas prunicola* antigen, which are evidently an error in the final manipulation, are in agreement that *P. prunicola* is not to be distinguished from the two pear-blast isolates, Receptacle and Lincoln. This is equally evident in the four different groups of tests, whether it be where *Erwinia amylovora* isolates are used as antigens and *P. prunicola* as antisera, *Er. amylovora* as antisera and *P. prunicola* as antigens, the pear-blast isolates as antigens and *P. prunicola* as antisera, and the pear-blast isolates as antisera and *P. prunicola* as antigens. This was even more evident in the results of the final series in which five different fluorescent isolates, including *P. prunicola*, were compared. The table likewise shows that the *P. prunicola* of England and the pear-blast isolates of Arkansas produce antibodies which cross agglutinate, the titers being of the same order in homologous and heterologous tests. Thus, the antiserum of *P. prunicola* yielded even higher titers of agglutination against the pear-blast isolates than against itself.

The results in the first series show that *Erwinia amylovora* is a close, homogenous group, unrelated to the fluorescent group, and that in the latter *Phytomonas prunicola* is not to be distinguished from pear-blast isolates.

#### ORGANISMS USED IN SECOND SERIES OF AGGLUTINATION TESTS

A consideration of the literature previously presented clearly shows that *Phytomonas syringae*, *P. citriputeale*, *P. cerasi*, and *P. prunicola* are closely related as far as morphological, cultural, physiological, and pathological tests are concerned, although it is evident that the tests as a whole are far from exhaustive. Similar tests made by one of the writers confirm this conclusion. In addition, a number of other described "species" have been noted which apparently belong to the same group. An effort was therefore made to obtain all these organisms in order to compare them in morphological, cultural, physiological, pathological, and serological reactions. In addition to *P. prunicola*, the only other organisms that the writers were able to obtain were *P. syringae* and *P. citriputeale*. Fortunately, these three represent considerable diversity of hosts and of geographic distribution, *P. prunicola*, as already noted, coming from England and having been isolated from plum, and *P. syringae*, presumably from Illinois, with lilac as its host, and *P. citriputeale* from California, with citrus and avocado as its hosts.

In order to compare these with *Erwinia amylovora* and the pear-blast isolates from Arkansas they were similarly treated throughout, grown on various culture media, subjected to different temperatures, tested for pathogenicity (figs. 2, 3, 4, 5) on different hosts, and finally compared in serological reactions. With the exception of *Phytomonas citriputeale*, which had apparently lost its virulence, *P. syringae* and *P. prunicola* produced symptoms on pear, apple, orange, and lemon which were indistinguishable from those produced by the pear-blast isolates. (Figs. 1, 2, 3.) However, in view of the fact that the *P. citriputeale* isolate presented very comparable cultural and physiological reactions it was included in the serological tests.

As a further check on the first series of serological tests two of the isolates of *Erwinia amylovora* previously used were included,

Hlg 12/17/30 and Kieffer leaf spot, and another isolate, Jonathan limb, obtained in June, 1931, from a blighted Jonathan apple limb, was added. Two pear-blast isolates, different from those used in the first tests, were incorporated, Petal, representing an isolate from a diseased petal of a closed, blasted Kieffer pear blossom gathered

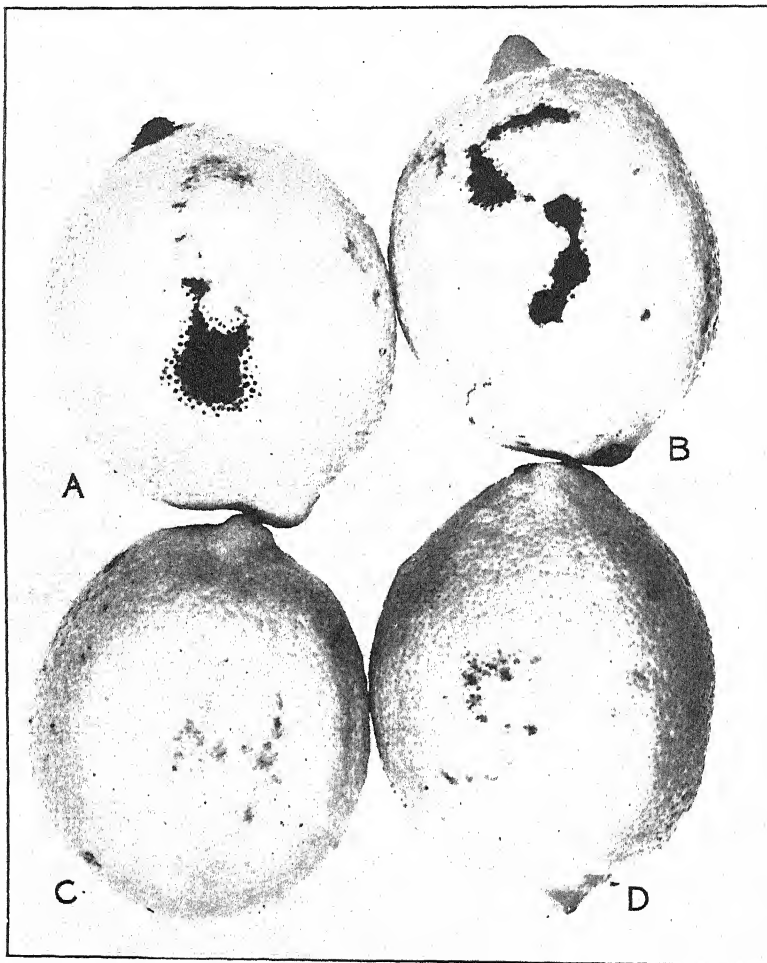


FIGURE 5.—Artificial inoculations on lemons with *Phytophthora syringae* (B); pear-blast isolate, Garber (A); *P. citripuleale* (D); and a fire-blight isolate, Hlg 12/17/30 (C). Note the infections with *P. syringae* and the pear-blast isolate, Garber, in contrast to the lack of infections with the nonvirulent *P. citripuleale* and the fire-blight isolate.

March 26, 1930, and Garber, representing a young blasted fruit of Garber pear gathered April 28, 1931.

The serological tests were conducted in the same manner as previously described with the exception that four intraperitoneal injections were made instead of five. This, however, is not recommended since the titers are as a whole lower than with five injections. (Compare Table 1 with Table 2.)

TABLE 2.—Titers of the second series of agglutination tests

Type of antigens	Titers of antisera indicated							
	Hlg 12/17/30	Jonathan limb	Kieffer leaf spot	Petal	Garber	<i>Phyto- monas syringae</i>	<i>Phyto- monas prun- icola</i>	<i>Phyto- monas citri- puteale</i>
Hlg 12/17/30.....	1-5, 120	1-1, 600	1-640	0	0	0	1-40	0
Jonathan limb.....	1-5, 120	1-5, 120	1-800	0	0	0	0	0
Kieffer leaf spot.....	1-1, 600	1-1, 280	1-1, 280	0	1-40	0	0	0
Petal.....	1-640	1-2, 560	1-800	0	1-10	1-40	0	1-20
Garber.....	1-1, 600	1-800	1-800	0	1-10	0	0	0
<i>P. syringae</i> .....	1-2, 560	1-1, 600	1-1, 600	0	0	0	0	0
<i>P. prunicola</i> .....	1-20	1-20	1-40	1-160	1-320	1-320	1-320	1-800
<i>P. citriputeale</i> .....	1-20	1-80	1-40	1-640	1-640	1-80	1-320	1-640
	1-40	1-40	1-20	1-640	1-800	1-640	1-320	1-640
	1-40	1-80	1-20	1-640	1-640	1-320	1-320	1-640
	1-20	1-80	0	1-40	1-320	1-320	1-320	1-320
	1-40	1-40	1-80	1-320	1-160	1-320	1-160	1-80
	1-20	1-20	0	1-320	1-160	1-320	1-320	1-320
	1-40	1-80	1-40	1-640	1-320	1-160	1-320	1-640
	0	1-10	0	1-160	1-80	1-1, 280	1-640	1-800
	0	0	0	1-320	1-80	1-1, 280	1-640	1-640

Table 2 again shows the close relationship existing between the various isolates of *Erwinia amylovora*, the cross-agglutination tests showing titers as high as in the homologous sera antigens. By combining these results with those of the first series it would appear that, irrespective of the kind of host, of the kind of tissues used for isolation, or of the season of the year when the isolation is made, the isolates are so remarkably similar in these serological tests as to suggest an old firmly fixed, almost immutable species. It hardly seems possible that this will hold true for any large number of bacterial plant pathogens, and it is to be noted that all the isolates came from but one State.

There is no apparent relationship shown between *Erwinia amylovora* and the 5 fluorescent isolates. Of the 60 linear series of cross agglutinations between the 3 isolates of *Er. amylovora* and the 5 fluorescent ones, 32 show no agglutination whatever and the remainder show that of group titer.

On the other hand, the 5 fluorescent isolates, while evidently not so homogenous as the isolates of *Erwinia amylovora*, show a very close kinship to each other. In these serological investigations the two pear-blast isolates, Petal and Garber, are hardly to be distinguished from *Phytomonas syringae*, *P. prunicola*, and *P. citriputeale*. Neither are the latter 3 to be distinguished from each other. While the titers of these are not so high as those in the *Er. amylovora* isolates, the results are so similar throughout that their relationship can be noted in any of the tests. The heterologous sera antigens present as high or even higher titers than the homologous ones. Thus Petal antiserum at a titer of 1 to 640 agglutinates Petal, Garber, and *P. prunicola*; *P. syringae* antiserum agglutinates at 1 to 320, the highest point of its own titer, Petal, Garber, *P. prunicola*, and *P. citriputeale*; *P. prunicola* antiserum agglutinates at 1 to 320 Petal, Garber, *P. syringae*, and *P. citriputeale*; and *P. citriputeale* antiserum agglutinates Petal at a titer of 1 to 800, and Petal, Garber, and *P. prunicola* at 1 to 640. Out of 50 different linear series of agglutinations in this whole group, 5 fail to show this relationship, and as the

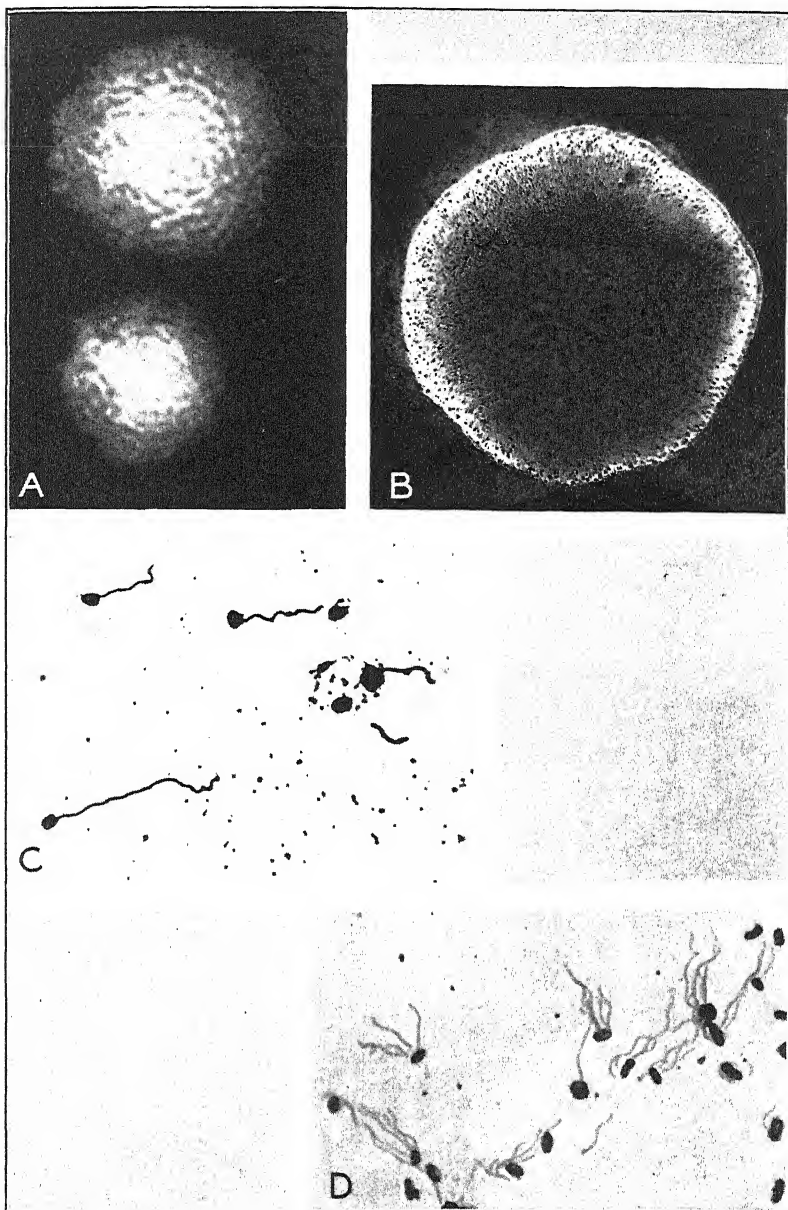
duplicates of 4 of these 5 show significant titers in the cross agglutinations the exceptions may be properly questioned. The fifth, Garber antiserum and *P. citriputeale* antigen, with two titers of only 80, are likewise to be discounted since the reciprocals run in duplicate, *P. citriputeale* antiserum and Garber antigen, present a titer of 1 to 640.

#### COMPARISON BETWEEN SEROLOGICAL REACTIONS, PATHOGENICITY, AND CULTURAL CHARACTERISTICS

The pathological and cultural tests, in which the senior writer, among other things, found that *Phytomonas prunicola*, *P. syringae*, and pear-blast isolates are equally pathogenic and produce similar symptoms on pears, apples, oranges, and lemons, the only hosts tried, fully confirm and amplify the serological investigations. There can be no question that from the practical point of view little or nothing is to be gained by considering them as different species. This does not preclude the possibility of some cultural, morphological, or physiological difference. They do exist in this fluorescent group to a far greater extent than in *Erwinia amylovora*. But this is not necessarily associated with any disparity in hosts. For example, the two pear-blast isolates, Receptacle and Petal, behave remarkably alike, no matter what the test may be, while the isolates, Lincoln and Garber, differ from these not only in amount and kind of pigment production but also in various other ways. Nevertheless, they were all originally isolated from pear, and all produce like symptoms in artificial inoculations. Since the difference between these is comparable to the difference in isolates on unlike hosts it appears evident that such differences can not be utilized in differentiating species within this group. To say that *P. prunicola* is a different species from *P. syringae* or from pear-blast isolates because it shows some differences would be equivalent to saying that nearly every other isolate from pear blast is a different species. Not only have variations been found in pear-blast isolates, but almost all the previous writers have noted similar differences in isolates from the same hosts in this same fluorescent group. Both Goldsworthy (14) and Wilson (45) made special studies of different isolates of *P. cerasi* and emphasized the differences in these when derived from the same host. Bryan (9) described a marked cultural difference between two isolates from lilac blight, one producing a rough, wrinkled colony, comparing with the feature described by Wormald (46) as characteristic of *P. prunicola*, and the other showing no wrinkling. She further found that agar colony characters of *P. citriputeale* were no more different from *P. syringae* than were isolates of the latter different from each other. Hopkins<sup>10</sup> likewise noted a marked difference in isolates from the South African bark disease of apples, some producing a fluorescent pigment and others not, and it has already been noted that Smith and Fawcett (43) found variation in the citrus-blast bacterium when it was grown for some time on culture media, changing from a smooth to a wrinkled growth and gradually losing its power to liquefy gelatin.

It is evident that within this group of fluorescent pathogenes there are not only disparities between different isolates from the same host but also that any one isolate may develop variations in morphology

<sup>10</sup> HOPKINS, C. J. Op. cit.



A, Typical colonies of bacteria, 2 days old, on beef-extract agar plates, isolated directly from a young, blasted Garber pear fruit. Transfers from individual colonies of this rough, irregular type produced typical blast symptoms on pear shoots. Compare with B.  $\times 14$ . B, Typical colony of bacteria, 2 days old, on beef-extract agar, representing a reisolation from an artificial infection on pear induced by the rough, irregular type of colony originally isolated from a blasted Garber pear fruit. Compare with A. Artificial inoculations with transfers from such single, smooth, definite-margined colonies produced infections directly comparable with those produced by the rough, indefinite-margined colonies.  $\times 14$ . C, *Erwinia amylovora* Hig 12/17/30 isolate, stained to show flagella. Note the polar arrangement and compare with D.  $\times 1,400$ . D, *Erwinia amylovora* Hig 12/17/30 isolate, stained to show flagella. Note the peritrichic arrangement and compare with C.  $\times 1,400$



and in cultural (pl. 1, A and B), physiological, and pathological behavior at different times. In other words, contrasted with *Erwinia amylovora*, the fluorescent pathogenes here considered represent an elastic, highly variable group in which species delimitation on the basis of disparity in hosts or in any other recognized standard of comparison appears uncertain, and in any large measure, almost incapable of exact duplication. A carefully evolved description of any isolate or group of isolates may not be applicable to the very same isolates at another time.

The degree of infectivity as measured by the size of lesions, virulence, and loss of virulence is of interest in this group. Apparently Smith and Fawcett (43) hesitated to place *Phytomonas syringae*, *P. cerasi*, and *P. citriputeale* in one species (*P. nectarophila*, *P. papulans* and *P. barkeri* were not considered, and *P. prunicola* and *P. utiformica* appeared after their publication), because artificial inoculation with isolates from different hosts did not always yield comparable sized lesions on lemons, although

the strain of *Bact. cerasi* from apricot and sweet cherry and of *Bact. syringae* from lilac when inoculated at room temperature by punctures in lemon fruits, developed typical black pit spots that were not distinguishable from those produced by *Bact. citriputeale*.

If any large number of isolates from citrus were studied alongside of an equal number from other hosts it is a question whether the citrus ones would always produce larger spots on lemons. The present writers have produced spots on both lemons and oranges at a temperature varying from 15° to 17° C. with pear-blast isolates, with *Phytomonas prunicola* and with *P. syringae* (figs. 4 and 5) which were fully as large as those found by Smith and Fawcett for *P. citriputeale*, and these isolates produced spots when *P. citriputeale*, owing to a loss of virulence, failed to produce any. If loss of virulence occurs in artificial cultures, is there any good reason for assuming that this does not occur at times in nature? Furthermore, the loss of virulence may not be complete, and the variation in virulence which has been noted by a number of investigators of this group of bacteria, notably by Barss (3) in *P. cerasi*, is clearly indicative of what may be expected with these fluorescent bacteria when a large number of isolates are carefully studied. As a matter of fact, when attempting to isolate a pathogene, investigators are likely to discard a culture that does not produce noticeable infections in artificial inoculations, on the supposition that it does not represent the pathogenic species. That this is not always a proper assumption has been amply shown by Patel (29) who found quite a number of nonvirulent strains of *P. tumefaciens* existing in nature. Burkholder (10) in speaking of bacterial plant pathogenes in general is obviously correct in stating that too many species have been described on the basis of pathogenicity to some host.

#### DISCUSSION AND CONCLUSION

The biologic reaction between a host and a given microorganism constitutes an important means of determining relationships between similar microorganisms, and it is far more specific than any commonly used cultural, physiological, or morphological test. Often, however, the reason cited for the erection of a new species is that the published accounts of some cultural or morphological characters do not fit the

organism at hand, but in only a few instances have the writers taken the trouble to verify the supposed differences.

It is to be questioned whether sufficient difference exists in either pathogenicity or in cultural and physiological reactions between *Phytomonas syringae*, *P. cerasi*, *P. citriputeale*, *P. papulans*, *P. nectarophila*, *P. barkeri*, *P. prunicola*, *P. utiformica*, and pear and apple blast isolates to justify their acceptance as distinct species. Sufficient serological evidence having been presented to show that *P. syringae*, *P. citriputeale*, *P. prunicola*, and four pear-blast isolates are alike, it would seem desirable to consider these at least as belonging to one species, and if the excellent pathogenic and cultural studies of Smith and Fawcett (43) on *P. cerasi* are accepted, the gummosis pathogenes would also be added. It would of course be desirable to include fluorescent pathogenes other than those already mentioned together with fluorescent saprophytes in serological studies. For the present the writers are accepting *P. syringae* as the oldest established name for this whole group of bacteria, but in view of the lack of serological evidence of a large number of other fluorescent bacteria this acceptance must be regarded as tentative. One thing seems certain with regard to these fluorescent bacteria that without serological tests the very close relationship existing between them is very likely to be underestimated. While numerous investigators have called attention to such relationship, it is questionable, judging by the array of "new species" that are continuously being described, whether its true significance has been fully realized.

From the taxonomic point of view, because of the lack of any widely accepted standards, it makes perhaps little difference whether one considers *Phytomonas syringae*, *P. cerasi*, *P. citriputeale*, *P. prunicola*, etc., as distinct species or varieties, races, strains, clons, or physiological forms of one species. The pragmatic question remains: Does the lilac-blight pathogene confine itself to lilac or does it also attack pear, apple, apricot, plum, avocado, citrus, and other hosts? If it does, and the evidence here presented substantiates this view, then what useful purpose is served in considering some isolates within this group as distinct species because of cultural or physiological disparity? When one considers the relatively large amount of evidence which is accumulating with respect to the variability or "dissociation" of bacteria, it would seem that, contrary to the rather common practice of considering a few differences as sufficient ground for separating species, such differences are to be expected in any one species. One need not accept the pleomorphism and almost endless variability theories of Löhnis (23, 24, 25), Enderlein (13), or Hadley (17, 18) to conclude that if no differences in any group of related isolates have been found there is good reason for supposing that a given study is incomplete.

While cultural and physiological reactions in the groups of *Erwinia amylovora* and *Phytomonas syringae* show close agreement, the serological tests show practically no relationship between the two species. It has already been noted that Wormald found no difference in the group numbers between his fluorescent *P. prunicola* and *Er. amylovora*, both having exactly the same number, 211.2322033, and the chief reason given by Wormald for not considering them to be the same species is the arrangement of flagella, a character which of all bacte-



riological morphology, with the possible exception of capsules, is with the present technics apt to be most evasive and uncertain. No flagella-staining technic known to the writers has as yet been devised which has met general acceptance, and nearly every laboratory has its own particular method. The senior writer has one which has been successfully used for a number of years and which has given some excellent results (33), but it has not been published and probably will not be for some years because there is at present no certainty that it will give comparable results to different investigators and that it will stain the flagella of all bacterial species equally as well. This method when used on fifty-odd isolates of *Er. amylovora* shows the flagella to be variable in any one preparation, but the greatest number by far show only polar flagella (pl. 1, C), and this is consistent with the results that have previously been published (32). Nevertheless a few organisms can almost always be found with peritrichic flagella (pl. 1, D). By using the Casares-Gil's (Plimmer and Paine) method Bryan (8) finds the flagella of *Er. amylovora* only in peritrichic arrangement, and some of her excellent preparations which have been made available to the senior writer fully confirm her findings. Unfortunately, however, the writers have been unable to duplicate her work by the Casares-Gil's method. The point to note is that, irrespective of the arrangement of flagella in *Er. amylovora*, there is no flagella-staining method available at present which can always be relied on to give comparable results to different investigators. Consequently any taxonomic system which at this time is based upon either number or arrangement of flagella is likely to be insecure. The inclusion of nonmotile and polar flagellated plant pathogenes in one genus, *Phytomonas*, as adopted by Bergey et al. (5) appears to be justifiable from this point of view, but the separation of *Erwinia* because of flagella arrangement rests on uncertain ground. If *Bacillus carotovorus* be considered the type of *Erwinia* rather than *Er. amylovora*, then, with the exception of a few anomalous species which are now included, the group as a whole might be classed as gas formers in carbohydrate media, in contrast with *Phytomonas*, which would include all nongas formers.

While there are undoubtedly a number of cultural and physiological dissimilarities between *Erwinia amylovora* and *Phytomonas syringae*, the number of points of agreement as previously set forth is perhaps as great as those within the group of *P. syringae* itself. Were it not for the marked difference in symptoms on pears, and the total absence of infections on citrus fruit, in the case of *Er. amylovora*, it would be difficult to harmonize the cultural and physiological similarities with the serological disparities. There can be no question that the so-called salient cultural and physiological tests fail to reveal the marked difference between the two groups. On the other hand, the agreement between the pathogenicity tests and the serological reactions is perhaps the very best evidence of the reliability of serological reactions in determining relationships among bacterial plant pathogenes.

The writers conclude that the disease of pears and apples called blast is caused by the same species of bacteria that produces lilac blight, gummosis of stone fruits, citrus blast and black pit, avocado blemish, blossom blight of pears, bacterial canker of apples, and bacterial wilt of plums.

## SUMMARY

Attention is directed to a disease of pears and apples hitherto unreported in North America, which simulates fire blight and which is caused by a fluorescent bacterial pathogene.

The disease, termed "blast," is noted as being most common in the early part of the growing season and as affecting closed and open flowers, leaves, and young fruits. In artificial inoculations it also involves blighting of tender twigs, with symptoms distinctly different from fire blight.

The pathogene shows, on the one hand, numerous cultural and physiological reactions identical with those of the fire-blight organism, *Erwinia amylovora*. On the other hand, it also in large measure presents characteristics that have been attributed to various fluorescent plant pathogenes, including *Phytomonas syringae*, *P. cerasi*, *P. citriputeale*, *P. papulans*, *P. nectarophila*, *P. barkeri*, *P. prunicola*, and *P. utiformica*.

A number of isolates of pear blast were compared with several *Erwinia amylovora* isolates, with *Phytomonas syringae*, *P. citriputeale*, and *P. prunicola* (the only ones available), the comparison consisting of practically all the common cultural and physiological tests, morphology, pathogenicity, and serological reactions.

This paper largely concerns itself with the serological investigations. Data concerning the pathogenicity tests are included, however, to indicate the similarity of these to the serological reactions.

*Phytomonas syringae*, the lilac blight organism of Europe and North America; *P. prunicola*, the English plum-wilt pathogene; and the American pear-blast isolates produce symptoms on pear twigs and leaves that are indistinguishable. These symptoms are obtainable with and without wounding. The only culture of citrus blast isolate, *P. citriputeale*, available to the writers had evidently lost its virulence. It produced no noticeable infections on lemons and oranges, though a reisolation from a very small lesion that it produced on a wounded pear twig yielded a culture which possessed a more noticeable pathogenicity for pear twigs. Pear-blast isolates, *P. syringae* and *P. prunicola*, produce symptoms on oranges and lemons that are indistinguishable from those produced by the citrus blast pathogene, *P. citriputeale*. *Erwinia amylovora* isolates, on the other hand, produced no infections on oranges and lemons.

The serological tests are found to be in full agreement with the pathogenicity studies. The pear-blast isolates show on one side no relationship to *Erwinia amylovora* and on the other side a very close kinship to *Phytomonas syringae*, *P. citriputeale*, and *P. prunicola*. Including the pear-blast isolates, these four are so closely related, as revealed in cross agglutinations, that they are indistinguishable. It is also concluded that, judging from the published accounts of *P. cerasi*, *P. papulans*, *P. nectarophila*, *P. barkeri*, and *P. utiformica*, these are probably also allied with the above and are of questionable standing as distinct species. For the present the name *Phytomonas syringae* is accepted for this group.

The significance of cultural, physiological, morphological, pathological, and serological studies in differentiation of species is discussed.

Since the current cultural and physiological tests are largely responsible for the multiplication of binomials of bacteria that are

otherwise shown to be closely related, and since the cross-inoculation tests on the different hosts involved in the fluorescent bacteria under consideration show little or no host restriction, it is contended that no useful purpose is served in erecting new species on the basis of a few cultural and physiological differences or on assumed host relationship.

The noteworthy correlation shown between pathogenicity and serological reactions is emphasized as indicative of the value of serological investigations for determining relationships of bacterial plant pathogens.

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# CHEMICAL TRANSFORMATIONS OF PHOSPHORUS IN THE GROWING CORN PLANT, WITH RESULTS ON TWO FIRST-GENERATION CROSSES<sup>1</sup>

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## INTRODUCTION

In the course of cooperative corn-disease investigations carried on by the Division of Cereal Crops and Diseases of the United States Department of Agriculture and the Department of Agronomy of the University of Illinois, several inbred strains of yellow dent corn, *Zea mays indentata*, have been developed, which, after a number of generations (8 to 12) of inbreeding and selection, have become practically uniform in most visible or external characteristics, and perhaps somewhat less uniform in certain functional or internal characteristics, notably their reactions to some of the disease-producing organisms and to exposure to low and high temperatures. However, it was observed that when these inbred strains were planted on differently treated soils the responses to fertilizer treatment were distinctly lacking in uniformity among the different single-ear progenies within a given inbred line, among the different plants of many single-ear progenies, and also among certain first-generation crosses. These differences were particularly striking in the case of response to phosphate fertilizer applications. It was at once apparent that an understanding of the physiological and chemical aspects of these differences in nutritional capacity within the same species would add to the knowledge of plant metabolism, and might also contribute to the practical end of more advantageous use of fertilizers and better adaptation of corn varieties or strains to soil environment.

The work of producing the strains used and their behavior in the field will not be discussed in the present paper. This paper is concerned with a preliminary study of the distribution of various chemical forms of phosphorus in the corn plant at various stages of its development, and the distribution of the total phosphorus among four groups of compounds, organic and inorganic, in two first-generation crosses.

## TRANSLOCATION AND CHEMICAL TRANSFORMATIONS OF PHOSPHORUS IN THE GROWING CORN PLANT

The vegetative period in the life of the corn plant is concerned primarily with the acquisition of inorganic substances from the soil and air, and their transformation into organic materials. During the reproductive period this process gradually gives way to a general

<sup>1</sup> Received for publication July 10, 1931; later withdrawn for the inclusion of additional data; issued February, 1933. This paper is an outgrowth of studies first reported in the division of agricultural and food chemistry of the American Chemical Society at its Indianapolis meeting, Apr. 1, 1931. The work reported was done in the soil fertility laboratory, Department of Agronomy, University of Illinois.

movement of these more or less elaborated materials into the developing seeds in which is concentrated at maturity a reserve of carbohydrate, protein, oil, and other organic compounds. Of the total phosphorus content of the corn plant at maturity, approximately three-fourths is to be found in the seeds; and of the portion present in the seeds, three-fourths or more occurs in the form of phytin. Values reported by Webster (44)<sup>2</sup> place the phytin phosphorus at 82.6 per cent of the total phosphorus in yellow dent corn, and 76.5 per cent in a white dent variety. Values obtained by the writers in yellow dent samples range about 83 per cent. That phytin is an important storage form of phosphorus in seeds is indicated by the relatively large amounts present in the seeds of various species. In the 11 different species reported by Webster, an average of 65 per cent of the total phosphorus was in phytin, and in only 2 species was less than 50 per cent of the total phosphorus present in this compound.

The elaboration of phosphorus compounds during the vegetative period and the translocation and storage of this element in the seeds during the reproductive stage are processes in which may be found the basis of the differential response to this element of various strains of corn such as those previously referred to. Another possible cause of the observed variations in behavior toward phosphate applications is the ability of the plants to secure the phosphorus needed for growth from the compounds present in the soil and in the soil solution. This aspect of the problem with reference to various species of plants has been emphasized in discussions of absorption by plants, or the so-called "feeding-power" of plants, by Truog (41, 42, 43), Davis, Hoagland, and Lipman (20), Newton (33), and others.

The weight of experimental evidence indicates the general absence of phytin in the vegetative parts of growing plants. Hart and Tottingham (23) were unable to find evidence of its presence in rutabagas or in alfalfa hay, cut in the early bloom stage. Anderson (2, 3, 4, 5, 6, 7, 8, 9, 10, 11), in a series of investigations on the chemical nature of phytin, extending over about 10 years, described its isolation from seeds and seed products of many plants, but did not report its isolation from vegetative parts of plants. The work of Rather (37) and of Averill and King (15) falls in the same category with Anderson's in this respect. One exceptional case may be noted. Henrici (24) made an intensive study of the phosphorous compounds and their distribution in numerous grasses. These grasses were collected in South Africa, where they had grown in phosphorus-deficient soils under climatic conditions which were generally semiarid. Where the rainfall was about 18 inches annually, no phytin or water-soluble phosphorus was found in leaves of most species. At Ermelo, however, where the rainfall was 36 inches, leaves of *Eragrostis abyssinica*, collected in 1926, contained 0.022 per cent of phytin  $P_2O_5$  by the Heubner-Stadler (26) method, and 0.047 per cent of water-soluble  $P_2O_5$ . Young leaves of *Monocymbium ceresiiforme*, taken before jointing occurred, contained 0.061 per cent of water soluble  $P_2O_5$ , and the phytin determination gave the same value. Later in the season when seed stalks had formed, the values in old leaves had fallen to two-thirds the original values, but still remained essentially identical in the two determinations, namely, 0.039 and 0.40 per cent

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 139.



for phytin  $P_2O_5$  and water soluble  $P_2O_5$ , respectively. This is the only instance, with supporting data, which the authors have found of the presence of phytin elsewhere than in developing or mature seeds or closely associated organs, such as pericarps. Kostychev (31, p. 215-216), makes the following statements:

Perhaps phytin or inosite phosphoric acid is to be regarded as the first product of the assimilation of phosphorus. \* \* \* During the ripening of seeds, a considerable amount of phosphorus enters the seed in the form of inorganic phosphates and phytin and there are formed all the compounds of phosphorus. These are the facts concerning the transformations and movements of phosphorus compounds on the basis of thorough investigations by [W] Zaleski.

The original work of Zaleski has not been available. Scattered statements of the occurrence of this form of phosphorus in leaves or stems of plants have been seen in the early literature, but have not been accompanied by experimental data.<sup>3</sup>

The first experiments of the present authors were undertaken to determine the presence or absence of phytin phosphorus in different parts of growing and maturing corn plants. For this purpose, a qualitative test was considered adequate. The test in each case was carried out as follows:

The fresh plant material was macerated and then extracted with 2 per cent HCl. The filtered extract was then diluted to 0.6 per cent concentration of HCl, and  $FeCl_3$  dissolved in 0.6 per cent HCl was added. The criterion used was the formation or nonformation, as a white flocculent precipitate, of the ferric salt of inosite phosphoric (phytic) acid.

Tests carried out on stalks, blades, and also on the forming tassels and ears, when present, at intervals during the prepollination period gave negative results in all cases. The first of these were made when the corn was about 3 to 4 feet high, and negative results continued until after pollination. The qualitative test indicated an abundance of phytin in developing seeds removed from the ear two weeks after pollination; but only negative results were obtained on shanks and cobs. Tests for total phosphorus on ignited samples of both shank and cob, however, gave copious precipitates of ammonium phosphomolybdate. In the case of a number of ears which were bagged, thus preventing the silks from being pollinated, tests of both shank and ear two weeks after pollination time revealed total phosphorus in abundance, but no phytin. In one bagged ear on which about a dozen scattered ovules had been fertilized, there was a small quantity of phytin in the cob outside of the few developing seeds, while within these scattered seeds, the phytin concentration was high. This was the only instance in which phytin was found in any part of the plants except developing or mature seeds.

The next experiments were carried out for the purpose of observing the enzymatic hydrolysis of phytin during the seed-germination period. Seeds of a yellow dent variety of corn were placed in a germination chamber. Aliquots consisting of 25 seeds each were withdrawn at successive 24-hour intervals, and subjected to determinations of phytin phosphorus by the Heubner-Stadler (26) method. The results are recorded in Table 1. The samples were exhausted at the expira-

<sup>3</sup> Since the preparation of the present manuscript, the paper by Knowles and Watkin (29) has been received, in which are reported amounts of phytin phosphorus up to 95 per cent of the total phosphorus in the vegetative parts of wheat plants which had been dried at room temperature.

tion of 144 hours. A progressive decrease in phytin is observed, which, by extrapolation of the curve, would presumably have exhausted the phytin in less than two weeks. A qualitative test for phytin made at about the same time on corn seedlings 4 weeks old, grown in soil in the greenhouse, gave negative results.

TABLE 1.—*Phytin phosphorus in germinating corn seeds after varying lengths of time in the germinating chamber*

[Total initial phosphorus in corn seeds, 0.313 per cent]

Period in moist germination chamber	Phytin phosphorus present—		Period in moist germination chamber	Phytin phosphorus present—	
	In dry corn	As percentage of the total phosphorus		In dry corn	As percentage of the total phosphorus
<i>Hours</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Hours</i>	<i>Per cent</i>	<i>Per cent</i>
0	0.277	88.5	96	0.213	68.0
24	.264	84.3	120	“.193	61.6
48	.264	84.3	144	“.175	55.9
72	.254	81.1			

\* Great difficulty was experienced in titrating in these last 2 determinations because of obscuring of the end point by soluble organic matter. (See Rather (37).) A method has since been devised by 2 of the authors which overcomes this difficulty and which is being published elsewhere.

These experiments indicate that phytin is not normally utilized by the corn plant as a form for transport, but is formed at or near the point of storage in the ripening of seeds, and during germination is hydrolyzed to its cleavage products, inositol, and phosphoric acid or the metallic salts of the latter, before movement into the young plant.

The accepted reversibility of enzyme reactions (see Bayliss (16, chapter 5)), for instance, considered with the undisputed enzymatic cleavage of phytin by phytase (1, 8, 18, 35, 40) leaves no doubt that phytin synthesis is an enzyme reaction. Furthermore, the fact that the beginning of this synthesis follows immediately after pollination and does not occur before pollination suggests an intimate association of activation of the zymogen with fertilization of the ovule. Inasmuch as extremely small quantities of an activator may be sufficient to set off a progressive enzyme reaction, it is not beyond the range of possibility that the pollen grain may serve as the carrier of this activator. In this connection it may be noted that Anderson and Kulp (12) have found that corn pollen contains nearly 1 per cent of inositol, one of the building stones of the phytin molecule, and 0.22 per cent of inorganic water-soluble phosphorous, but no phytin. The remainder of the 0.63 per cent of total phosphorus was found to consist for the most part of phosphatides.

Inasmuch as the absence of phytin from the vegetative parts of corn plants is established, steps for the separation of phytin in these parts in the analyses hereinafter reported have been omitted.

Table 2 records the total phosphorus content of individual plants of two strains of yellow dent corn grown in 1929. One of these, inbred A, is a parent of cross 365, to which reference is made later in this paper. The first-generation cross is not related to either of the two strains used in the major part of this investigation. While these data are inadequate for statistical analysis, they indicate a fairly high degree of

uniformity in phosphorus content among the individuals in a given strain when grown under similar conditions and sampled at the same stage of development.

TABLE 2.—Total phosphorus content (per cent) in tops and roots of an inbred (inbred A) and a first-generation cross, just before pollination, grown on superphosphate-treated soil, Shirley, Ill., 1929

Strain and plant No.	Percentage phosphorus in—		Ratio of percentage P in tops to percentage P in roots	Strain and plant No.	Percentage phosphorus in—		Ratio of percentage P in tops to percentage P in roots
	Tops	Roots			Tops	Roots	
Inbred A:				First generation cross:			
1.....	0.335	0.181	1.85	4.....	0.270	0.206	1.31
2.....	.330	a. 188	1.77	5.....	.260	.197	1.32
2a.....	.338			6.....	.274	.203	1.35
3.....	.322	a. 179	1.82	7.....	.268	.184	1.46
3a.....	.331			8.....	.268	.191	1.40
Mean.....	.331	.183	-----	Mean.....	.268	.196	-----

\* Compositied sample of 2 plants.

Table 3 gives the total phosphorus content of different parts of the plants of two first-generation crosses used in this study at three stages during the season of 1930. These figures indicate an increasingly rapid movement of phosphorus from roots to tops as compared to the rate of absorption from the soil, with the approach of the reproductive stage. They also show a rapid accumulation of phosphorus in the ear at the expense of that in the vegetative parts up to two weeks after pollination, the time at which the last samples were taken.

TABLE 3.—Total phosphorus (per cent) in different parts of corn plants of two first-generation crosses, at three stages in development, grown on untreated, phosphorus-deficient soil near Urbana, Ill., 1930

Strain and date of sampling and stage of growth	Percentage of total phosphorus in—				Strain and date of sampling and stage of growth	Percentage of total phosphorus in—			
	Root	Top	Shank	Ear		Root	Top	Shank	Ear
F <sub>1</sub> 365:					F <sub>1</sub> 680:				
July 23.....	0.230	0.227	-----	-----	July 23.....	0.165	0.230	-----	-----
Aug. 2 (pollination).....	.087	.228	-----	-----	Aug. 2 (pollination).....	.106	.258	-----	-----
Aug. 16.....	.061	a. 150	0.341	0.495	Aug. 16.....	.094	a. 191	0.288	0.479

\* Ear and shank removed at the last sampling; entire tops included in earlier samples.

Throughout the period of rapid growth during the five weeks or so preceding pollination, a gradual decline in phosphorus concentration takes place in the tops, as shown by the 1931 analyses reported in Table 4. This decline is a result of the production of plant tissue at a greater rate during this time than the rate of uptake of phosphates from the soil. Toward pollination time, phosphorus migration into the developing, but yet seedless, ear gets under way. This movement continues, with a resulting high concentration in the ears in the samples taken 10 days after pollination. At this stage there is an upward gradient from the roots through the stalks and the shanks to the ears. (Table 3.) This high concentration in the ear is manifested in a number of samples representing different strains and con-

ditions of growth, as shown in Table 5, in which an average of 0.5 per cent is present. Mature corn seeds in comparison contain only approximately 0.3 per cent. (Table 1.) It is significant that the height of phosphorus concentration is reached at a time when cellular development is at the maximum, and in advance of the maximum accumulation of organic reserves. Later this concentration is lowered by dilution as nonphosphorized materials are laid down in the endosperm. These observations are in harmony with the finding of Cocke-fair (17) that phosphorus is concentrated to the greatest extent in the regions of greatest metabolic activity. This concentration of phosphorus in the partially formed seeds soon after pollination, but before the laying down of starch is far advanced, is of interest in the light of Cocke-fair's suggestion of the functioning of phosphorus in the synthesis of starch.

TABLE 4.—Trend, at successive stages of development, of total phosphorus concentration (per cent) in the aboveground portion of corn plants of eight different strains grown on phosphorus-deficient soil, with and without superphosphate fertilizer, near Urbana, Ill., planted June 7, 1931

Designation of strain	Total phosphorus in samples collected on—				
	July 6	July 18	Aug. 1	Aug. 11 <sup>a</sup>	Aug. 22
1.....	0.304	0.284	0.225	0.180	0.177
2(F <sub>1</sub> 365).....	<i>b</i> .322	<i>b</i> .284	<i>b</i> .252	<i>b</i> .203	<i>b</i> .192
3.....	.313	.276	.225	.154	.191
4.....	.296	.332	.253	.221	.204
5.....	.341	.334	.240	.182	.194
6.....	.376	.368	.259	.230	.179
7(F <sub>1</sub> 680).....	<i>b</i> .408	<i>b</i> .370	<i>b</i> .279	<i>b</i> .212	<i>b</i> .153
8.....	.404	.292	.252	.241	.186

#### SUPERPHOSPHATE

1.....	0.365	0.349	0.268	0.206	0.174
2(P <sub>1</sub> 365).....	<i>b</i> .346	<i>b</i> .295	<i>b</i> .278	<i>b</i> .206	<i>b</i> .186
3.....	.376	.288	.262	.203	.195
4.....	.403	.328	.288	.211	.221
5.....	.355	.292	.257	.191	.192
6.....	.381	.327	.264	.266	.226
7(P <sub>1</sub> 680).....	<i>b</i> .422	<i>b</i> .330	<i>b</i> .283	<i>b</i> .215	<i>b</i> .181
8.....	.431	.297	.267	.231	.181

<sup>a</sup> This sample taken at about the pollination stage. This and the next set of samples, taken Aug. 22, represent tops with the shoots or young ears removed.

<sup>b</sup> Compare sum of fractions in these samples, Table 6.

TABLE 5.—Total phosphorus content (per cent) of shanks and ears of eight samples of corn grown on untreated soil which was probably not phosphorus deficient, near Urbana, Ill.

[Sampled August 13, 1929, approximately 10 days after pollination]

Sample No.	Description of corn	Total phosphorus content of—	
		Shank	Ear
P 1359-60	Cross No. 598, slightly diseased; treated with Semesan Jr.....	0.356	0.457
P 1361-62	Cross No. 573, grown from seed from parent plants not frosted.....	.286	.583
P 1363-64	Cross No. 573, grown from seed from frosted parent plants.....	.289	.478
P 1365-66	Cross No. 365, grown from seed removed from parent ear before maturity.....	.349	.454
P 1367-68	Cross No. 365, grown from seed removed at maturity from the same ear as P 1365-66.....	.333	.536
P 1369-70	Cross No. 573, grown from seed removed from parent ear before maturity.....	.297	.478
P 1371-72	Cross No. 573, grown from seed removed at maturity from the same ear as P 1369-70.....	.314	.506
P 1373-74	Double cross No. 517.....	.365	.497

## CHEMICAL TRANSFORMATIONS OF PHOSPHORUS IN TWO FIRST-GENERATION CROSSES

## DESCRIPTION OF PLANT MATERIAL

Two first-generation crosses were used in this study, cross 365 (A $\times$ L) and cross 680 (A48 $\times$ A956). First-generation cross 365 had been recognized in previous field studies as exhibiting a pronounced response to phosphate treatment, indicated particularly by earlier maturity and also by increased yield. First-generation cross 680, on the other hand, had failed to respond appreciably to additions of phosphate fertilizers. The contrasting influence of phosphate fertil-



FIGURE 1.—First-generation crosses Nos. 680 (A) and 365 (B) growing on untreated soil. The corn shown in Figures 1 and 2 was planted the same day and the photographs were taken the same day

ization on the growth of these two crosses is shown in Figures 1 and 2, in which relative advancement in the stage of growth is the most outstanding difference. These crosses were grown on a phosphorus-deficient soil, both without fertilizer treatment and with the addition of 20 per cent superphosphate, applied and mixed with the soil around the hill at the rate of 250 pounds an acre just before the seed was planted.

Plant samples were taken for analysis at three different stages in 1930. Plantings were again made of these same strains<sup>4</sup> in 1931, in another part of the same field, on similar soil and with treatments the same as those used in 1930. In addition to sampling at the three stages represented by the 1930 samples, two other sets of samples were taken at earlier stages in the growth of the plants.

<sup>4</sup> The seed used in 1931 was produced by hand crossing the same parent inbreds, respectively, as were used the previous year, but after another generation of selection and selfing.

## FRACTIONATION OF THE PHOSPHORUS COMPOUNDS

A procedure was worked out for separating the phosphorus in these samples into four fractions, making use, so far as possible, of methods already published, as noted in the following discussion. The frac-



FIGURE 2.—Continuation of the rows shown in Figure 1 across an adjacent plot treated with superphosphate at the rate of 250 pounds an acre. A, cross No. 680; B, cross No. 365

tions were as follows: (1) The portion soluble in absolute alcohol, consisting mostly of lecithin together with other phospholipids; (2) inorganic phosphates; (3) acid-soluble organic phosphorus, which includes hexose-phosphate esters and possibly some easily soluble nucleoproteins, and (4) acid-insoluble organic phosphorus compounds,

chiefly insoluble nucleic acids, and insoluble nucleoproteins which are not readily hydrolyzed.<sup>5</sup> Groups 3 and 4 would also include any soluble and insoluble phosphoproteins, respectively, which might be present. Phosphoproteins, however, are not thought to exist in plant tissue (Osborne (34)).

These separations are somewhat arbitrary. Nevertheless they accomplish a division into rather well-defined groups, on a basis of chemical properties, and also to some extent with respect to plant functioning.

All four of the determinations were made upon the same sample, the last fraction, insoluble organic phosphorus, being the total phosphorus in the residue left from the last extraction. Consequently, the total phosphorus as determined in the original material should equal the sum of the four fractions. Determinations of total phosphorus have been carried out on samples of the materials used for fractionation in the work here reported, as well as on a considerable number of other samples, with satisfactory agreement. The results of total phosphorus determinations in F<sub>1</sub> 365 and F<sub>1</sub> 680, as given in Table 4 are comparable with the sums of the fractions in Table 6.

TABLE 6.—Percentage of phosphorus, separated into inorganic and three organic fractions in corn plants of two first-generation crosses, at five stages of development, grown on phosphorus-deficient soil, both with and without superphosphate fertilizer, near Urbana, Ill.; planted June 7, 1931

[Samples of July 6 and 18 and Aug. 1 represent entire aerial portion; those of Aug. 11 and 22 had ears removed]

#### CORN GROWN ON UNTREATED SOIL

Strain of corn	Phosphorus fraction	Percentage of phosphorus in samples collected on—									
		July 6		July 18		Aug. 1		Aug. 11 <sup>a</sup>		Aug. 22	
		Dry plant material	Total amount	Dry plant material	Total amount	Dry plant material	Total amount	Dry plant material	Total amount	Dry plant material	Total amount
F <sub>1</sub> 365.	Alcohol soluble.....	0.033	10	0.035	13	0.034	15	0.017	9	0.021	12
	Acid soluble organic....	.191	60	.124	47	.067	29	.064	34	.042	24
	Acid insoluble organic....	.027	9	.029	11	.046	20	.043	23	.044	25
	Inorganic.....	.068	21	.077	29	.085	36	.063	34	.070	39
	Total.....	.319	100	.265	100	.232	100	.187	100	.177	100
F <sub>1</sub> 680.	Alcohol soluble.....	.082	20	.036	10	.032	12	.028	14	.025	14
	Acid soluble organic....	.162	41	.148	41	.078	29	.050	25	.054	31
	Acid insoluble organic....	.044	11	.051	14	.056	21	.048	24	.043	24
	Inorganic.....	.110	28	.128	35	.104	38	.074	37	.054	31
	Total.....	.398	100	.363	100	.270	100	.200	100	.176	100

#### CORN GROWN ON SUPERPHOSPHATE-TREATED SOIL

F <sub>1</sub> 365.	Alcohol soluble.....	0.041	12	0.035	11	0.037	14	0.032	15	0.024	13
	Acid soluble organic....	.147	43	.122	38	.091	34	.051	25	.051	28
	Acid insoluble organic....	.053	15	.049	15	.049	18	.041	20	.052	29
	Inorganic.....	.101	30	.117	36	.090	34	.082	40	.053	30
	Total.....	.342	100	.323	100	.267	100	.206	100	.180	100
F <sub>1</sub> 680.	Alcohol soluble.....	.063	15	.036	11	.041	15	.020	9	.030	17
	Acid soluble organic....	.179	44	.127	40	.101	37	.051	24	.052	30
	Acid insoluble organic....	.051	13	.034	11	.041	15	.042	20	.039	22
	Inorganic.....	.113	28	.119	38	.091	33	.100	47	.053	31
	Total.....	.406	100	.316	100	.274	100	.213	100	.174	100

<sup>a</sup> Pollination stage.

<sup>b</sup> All of the phosphorus in fraction 4 can be brought into solution by refluxing with the 2 per cent hydrochloric acid 1, leaving a phosphorus-free residue.

The procedure used in separating the phosphorus into the four fractions is as follows:

(1) Phospholipids. Place 5 g<sup>6</sup> of dried, finely ground material in a Soxhlet extraction thimble and extract continuously with absolute alcohol for 8 to 10 hours. Remove the Erlenmeyer flask containing the extract, add to it 10 ml of 40 per cent magnesium nitrate solution and evaporate to a sirupy consistency on the steam bath. Transfer to a hot plate and heat at 180° C. until decomposition ceases. Then place in a muffle furnace heated just below redness until nitrogen oxides cease to be evolved and the residue is white. Remove from muffle, cool, take up with dilute nitric acid, and determine phosphorus.

(2) Inorganic phosphorus. Completely dry the residue from the previous alcohol extraction. Transfer to a 500-ml Erlenmeyer flask and add 300 ml of 95 per cent alcohol containing 0.2 per cent by weight of hydrogen chloride gas. Stopper the flask tightly and allow to stand for 3 to 5 hours with occasional shaking. Then decant most of the alcohol through a filter into a beaker. Wash the residue thoroughly with more acid alcohol by decantation. Return the portion of the residue on the filter paper to the main portion in the flask and save. Make filtrate slightly alkaline with concentrated  $\text{NH}_4\text{OH}$ . The phosphates precipitate out as a light, flocculent precipitate. Let stand overnight and then decant through a filter, without washing. Dissolve the residue on the filter with dilute nitric acid, allowing the liquid to run into the original beaker which contains most of the precipitate. When the precipitate has entirely dissolved, determine phosphorus as usual. This is essentially the procedure recommended by Collison (19). The acid alcohol solution is sufficiently acid to extract all inorganic phosphates, but without appreciable hydrolysis of organic phosphorus compounds.

Various other methods for the determination of inorganic phosphorus have been proposed by Posternak (36) and others. These methods consist in the main of precipitation with ammonium molybdate in the cold with the solution only slightly acid. Collison has objected to this procedure on the ground that even a weakly acid aqueous solution of nitric acid will produce hydrolysis of some types of organic phosphorus compounds. Moreover, a large amount of proteinlike material is precipitated, making filtration difficult. The method recommended by Collison, as above outlined, is, on the other hand, very clean cut. Both his results and the authors' experience with it appear to justify its use.

(3) Soluble organic phosphorus. To the residue from the acid alcohol extraction, add 150 ml of 2 per cent hydrochloric acid. Stopper the flask and allow to stand with frequent shaking for three hours. Filter, using a Büchner funnel with suction, wash the residue thoroughly with water, and save for (4). Transfer the filtrate with washings to a beaker. Add 7 ml of 40 per cent magnesium nitrate solution and evaporate to dryness on a steam bath. Then heat on the hot plate until fumes cease to be evolved and the residue is white. It should not be necessary to use the muffle. Dissolve in dilute nitric acid and complete the phosphorus determination in the usual way. Preliminary experiments by one of the authors showed

<sup>6</sup> g is the abbreviation for gram or grams recently adopted by the Style Manual for United States Government printing.



that the 3-hour digestion at room temperature with the 2 per cent hydrochloric acid used in separating groups 3 and 4 brought to completion the extraction of the substances which could be removed under these conditions, inasmuch as other periods of extraction up to 12 hours gave the same results. This separation has been used by Anderson (8), Rather (37), and Averill and King (15). Kostychev (31) uses water extraction for the readily soluble fraction, presumably after making the alcohol-ether extraction for lipids, according to Schulze and Steiger (39), subsequently precipitating out the inorganic fraction by means of magnesia mixture. This scheme would involve the difficulties mentioned above in connection with Posternak's procedure.

(4) Acid-insoluble organic phosphorus. The residue from the 2 per cent HCl extraction is dried, oxidized by the procedure of Howk and DeTurk (27), and subjected to total phosphorus determination. The method used for estimating phosphorus in the various fractions after destruction of organic matter consists of solution of the ammonium phosphomolybdate precipitate in standard alkali and titration of the excess alkali by means of standard acid.

#### COLLECTION AND PREPARATION OF SAMPLES

Each sample was a composite consisting of 7 to 10 plants growing in hills of 2 and 3 plants each. An effort was made to select plants as far as possible at the same stage of development. The stage of development represented by the different samples necessarily varied to some extent because of the differences in this respect in the two strains on any given date, especially on the phosphate plot. In 1930, the first two samplings (July 23 and August 2) represented the entire above-ground portion of the plants. The last samples, taken August 16, had the young ears removed. In 1931, the samples of the first three sets represented the entire aboveground portion but in the fourth and fifth sets the ears had been removed.

In the first season's fractionations (1930) the samples were brought to the laboratory, the roots washed as free of soil as possible, and after the roots had been separated from the tops, the latter were cut in lengths of 8 to 12 inches. The material was placed in a steam-heated drying oven at a temperature of 80° to 90° C. Ventilation was secured by partly opening the doors, before which electric fans were placed. Drying progressed gradually, 24 to 28 hours being required for completion. These fractionations on both strains, grown on phosphated as well as on untreated soil, gave results similar to those shown in Figure 3. The outstanding feature of these curves is the marked reduction of acid-insoluble phosphorus accompanying the rise in the acid-soluble fraction, at a stage in growth at which it could scarcely be accounted for by dilution due to increase in total dry substance. This distribution of the phosphorus fractions thus suggests a breakdown and reutilization of complex phosphorus compounds, such as nucleoproteins, nucleic acids, etc., which have been laid down as essential constituents of the cell substance. The possibilities of the occurrence of such a phenomenon will be discussed in a later paragraph. The evidence of the literature, however, lends but scant support.

Before the next season's work was begun the drying ovens were reconstructed in such a way as to produce rapid circulation of air by means of a forced-draft blower, without essentially altering the work-

ing temperature, except for a lowering of about  $10^{\circ}$  C. at the start, brought about by rapid evaporation. Experiments not reported here were carried out on divided samples, half dried gradually and half dried rapidly in the rebuilt oven. The results showed marked differences in distribution of the total phosphorus among the different fractions under the two conditions, indicating the necessity of early arrest of enzyme activity if extensive chemical changes in the samples are to be avoided. It was also found that splitting the stalks permits much more rapid drying than cutting them crosswise, even in very short lengths.

#### RESULTS AND DISCUSSION

In 1931 results, which are given in full in Table 6 and presented graphically in Figures 4 and 5, were obtained by analysis of samples

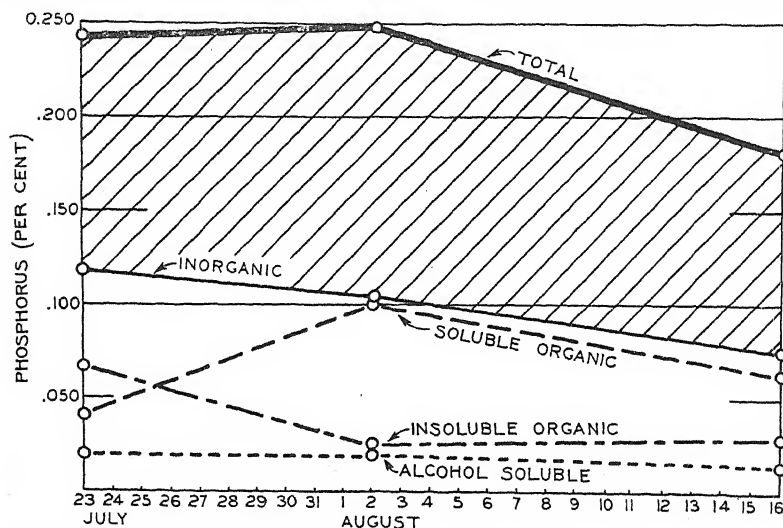


FIGURE 3.—Total phosphorus and its distribution among four fractions in a first-generation cross (F<sub>1</sub> 680) grown in 1930 without fertilizer treatment and dried slowly in preparation for analysis

in which the stalks were quartered lengthwise and the ears cut into small pieces, the whole being placed immediately in the ovens in open wire baskets. The drying temperature during the first hour ranged from  $70^{\circ}$  to  $80^{\circ}$  C., after which it rose to  $80^{\circ}$  to  $90^{\circ}$ . The drying was accomplished rapidly, approximately 25 per cent of the total water present being removed in the first 15 minutes, and 50 to 80 per cent in the first hour. It is believed, therefore, that the rapid drying, at temperatures at which the rate of enzyme destruction or inactivation exceeds the rate of its catalytic activity, has been effective in preserving the samples essentially unchanged. (See Gortner (22, chapter 25) and Collatz and Bailey (18).)

It will be observed (fig. 4) that the four phosphorus fractions fall into two groups. On the one hand are the nucleoproteins and related compounds (insoluble organic residue) and phospholipids (alcohol-soluble substances) which comprise an approximately constant part of the plant tissue throughout the growing season. These make up the more complex forms of phosphorus, the chemical la-

bility of which is very low. They are little if at all subject to translocation after having once been laid down in the cells. If any translocation occurs, the data indicate that it is in the lecithin fraction. As some of the other forms of phosphorus move into the ear in the later stages, leaving a lowered total concentration in the vegetative parts, the ratio of insoluble organic to total phosphorus rises (Table 6) because none of this fraction has moved out. In fact, the rise begins as early as August 1, during the period of rapid plant growth before pollination, when total phosphorus concentration is being reduced through dilution by newly synthesized material, thus indicating that the rate of formation of this fraction is keeping pace with plant growth. In the case of lecithin no rise in the ratio to total phosphorus is observed. Thus there is an indication that a portion of it may be broken down for transfer into the ear. The same possibility has been suggested by Eckerson (21), who found that in the tomato plant under conditions of phosphorus starvation the order of disappearance from older tissues is inorganic phosphate, starch-bound phosphorus, and phospholipids. The last named did not break down until most of the starch had disappeared. She says: "Although it is not certain that this (lipid) phosphorus can be reutilized, it seems possible." Koch and Reed (30) in the study of *Aspergillus* found that in phosphorus starvation after the disappearance of all of the water-soluble inorganic and organic phosphorus, lecithin decreased, but no loss of the nucleoproteins was established. In Figure 5, A and C, the percentages of lecithin and insoluble organic phosphorus have been combined and plotted as "nonlabile" phosphorus along with the other two groups, shown as "labile" phosphorus. The minimum requirement of the vegetative plant for the nonlabile material is shown by these data (figs. 4 and 5, A and C) to be a definite concentration which must be reached regardless of the nutritional environment. The concentration is essentially the same not only at the different growth stages but also in plants grown with either a deficient or abundant phosphate supply. Phosphorus absorbed in so-called luxury consumption is evidently not converted into these forms.

The other two fractions are characterized by marked chemical reactivity. These are the inorganic phosphates and the acid-soluble organic phosphorus. In the period covered by the first three samplings, no information is furnished on phosphorus movement because each sample includes the entire aerial portion of the plants. The rapid fall in concentration of acid-soluble organic phosphorus during this period must, then, be explained by its conversion to the nonlabile forms to meet the requirements of the rapidly expanding plant. (Fig. 4.) The absorption of inorganic phosphorus from the soil keeps pace at first, as shown by the rising concentration, but at the steepest part of the growth curve<sup>7</sup> it falls behind, as is seen in the change to a downward slope of the inorganic curve at the second sampling period in three cases, and at the third period as shown in Figure 4, A. The continued decline in these two constituents after pollination, shown, combined, as "labile" phosphorus in Figure 5, points to them as the source of phosphatic material for deposition

<sup>7</sup> The general character of the growth curve is shown by the superimposed curves for two of the four cases in fig. 4, B and C.

in the maturing seeds, as well as for formation of the stable lipid and nucleic phosphorus compounds in vegetative parts.

The data of the present investigation (fig. 4 and Table 6) do not prove that the soluble organic fraction is actually transported with-

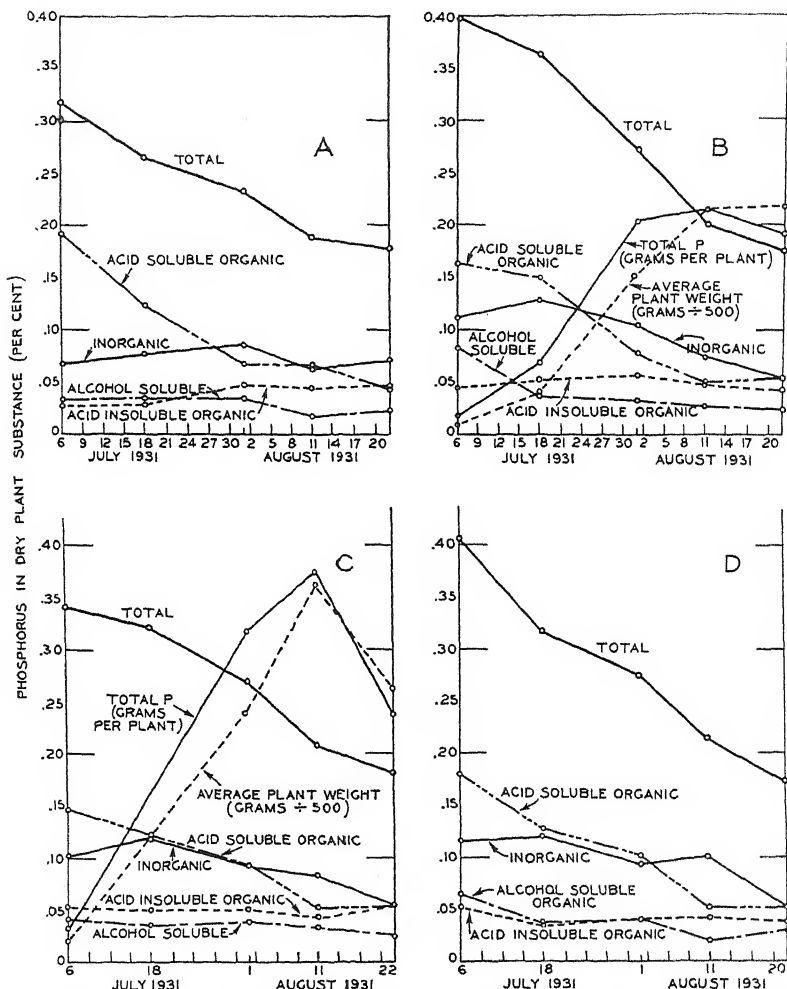


FIGURE 4.—Total phosphorus and its distribution among four fractions in two first-generation crosses of yellow dent corn. A and B give the values for F<sub>1</sub> 365 and F<sub>1</sub> 680, respectively, grown on phosphorus-deficient soil without fertilizer treatment; C and D give the values for the same crosses, respectively, grown on soil treated with superphosphate at the rate of 250 pounds an acre. In B and C are also shown the weights of the dry plants and the grams of total phosphorus per plant for two of the four cases. The marginal legend at the left applies also to these curves if the marginal notation be read "grams." The data are from Table 6.

out previous reconversion to the inorganic state. Either could be converted to the other in connection with the translocation.

The literature furnishes satisfactory evidence of the migration of phosphorus from tissues already formed into areas of new growth. For instance, the early investigations of Arendt (14) and of Wilfarth, Römer, and Wimmer (45) are cited. The results of Koch and Reed (30),

Eckerson (21), MacGillivray (32), Andre (13), Henri (24, 25), Reed (38), Jones and Huston (28), and others give further confirmation.

The question of the reutilization of phosphorus is not so easily settled. A discussion of this topic necessitates a definition of the term. As used here it will be understood to refer to the breakdown and translocation of those phosphorus-containing constituents of the cell which are essential to its functioning or to cell division. Thus nucleic acids, nucleoproteins and phospholipids will be included, represented

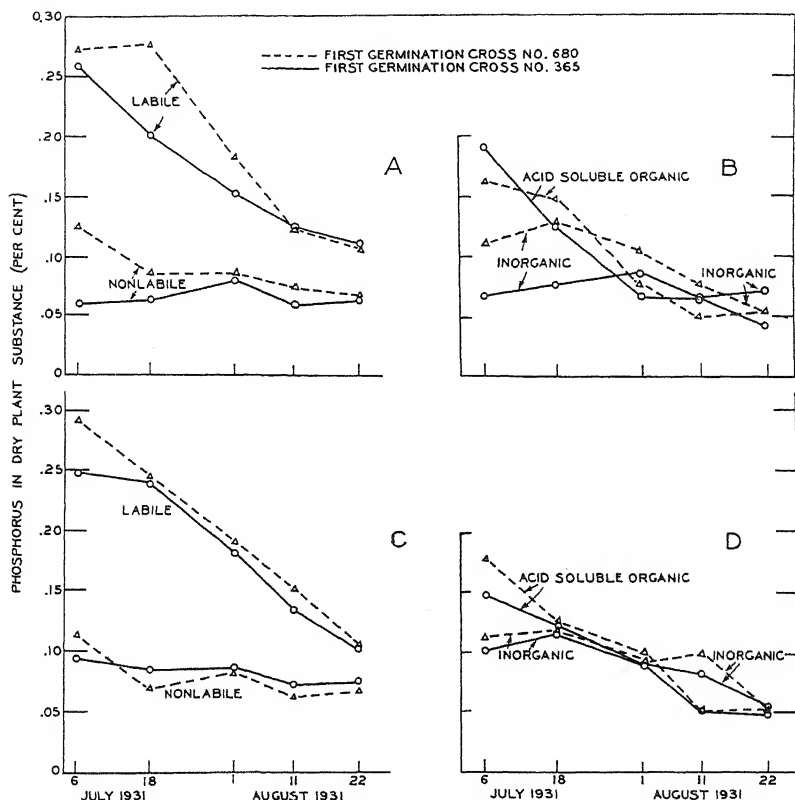


FIGURE 5.—Labile and nonlabile phosphorus in two first-generation corn crosses grown without (A) and with (C) superphosphate treatment. The labile fraction is the sum of the inorganic and the acid-soluble organic fractions. The nonlabile fraction is the sum of the alcohol-soluble and the acid-insoluble fractions. The two components of the labile fraction are shown separately for corn from untreated soil in B, and for corn from soil treated with superphosphate in D.

by groups 1 and 4 in the separations reported in this paper. The movement and use of the phosphorus-containing constituents of groups 2 and 3 including inorganic phosphates, hexose phosphates, and other soluble forms, as well as phytin, will not be considered as reutilization because these substances are not actual constituents of the living substance of the plant cell; they are merely present. Phytin is primarily a storage form and the inorganic and soluble organic compounds are of value chiefly for transport, the last-named probably constituting transition products in more complex syntheses as well.

The results of the present investigation and also a search through the literature have failed to reveal conclusive evidence of the reutilization of phosphorus. The results previously mentioned of Eckerson and of Koch and Reed with reference to phospholipids are the most suggestive but are not conclusive. MacGillivray (32) states in connection with his work on the tomato plant that "there is a reutilization of the phosphorus," and quotes André (13) as having shown a similar phenomenon. The phosphorus in MacGillivray's tomato plants, which was first carried into the lower leaves and later transferred to other parts may have consisted entirely of soluble compounds which had not become vital constituents of the cells of the lower leaves, whither they had first been transported. A classification of the phosphorus compounds would have been necessary in order to answer the question in this case. The facts shown by André were (1) that 100 beans contained 0.9828 g of total  $P_2O_5$  and (2) that in 100 plants approximately 30 cm long grown from similar seeds without nutrient salts, 26 per cent of the original 0.9828 g of  $P_2O_5$  was in the cotyledons and 67.3 per cent in the roots and tops, leaving 6.7 per cent unaccounted for. Webster's (44) analyses include several different kinds of seeds in which more than 74 per cent of the phosphorus is in phytin and inorganic combinations; i. e., storage and portable forms, not counting soluble organic phosphorus which he did not determine. No data on fractionations of phosphorus in garden beans have been found. In the absence of such data, and in view of the large amounts of transport and storage forms in seeds as shown by Webster, one would scarcely be justified in assuming that a breakdown and reutilization of the phosphorus of the protoplasm had occurred in André's experiment. The analyses of Henrici (24, 25) are of special interest in relation to this question. She carried out total phosphorus determinations at weekly, or in some cases fortnightly, intervals on the roots, crowns, leaves, jointed stems, and heads of 16 species of veld grasses and of some other plants found growing in the phosphorus-poor soils of South Africa near Pretoria. The analyses were continued for 12 to 13 months and parts of two other seasons. The yearly rainfall, varying from about 18 to 36 inches, was a limiting factor of growth in most cases. In another paper (24) she reported results of phosphorus fractionations on some of these grasses into essentially the same groups as those determined in this investigation. She found that in the regions of lowest rainfall, soluble phosphorus was absent or present in extremely small quantities in the leaves of most grasses. At Vryburg, with 18 inches of rain, no inorganic phosphorus was present in collective grass samples. In these cases the formation of heads was not accompanied by a decline in concentration of leaf phosphorus. The same was true at Armoedsvlakte with the same rainfall. Few species of the perennial grasses produced sound seeds under these conditions, and some produced no jointed stems. Two annual weeds, *Tragus* sp. and *Aristida* sp. growing in the same region always produced an abundance of viable seeds. Their leaves carried about the same percentage of total phosphorus as those of the grasses, but the heads reached 0.8 per cent. The practically seedless heads of grasses scarcely exceeded the low concentrations of the leaves (up to 0.3 per cent). Another characteristic plant of this semiarid region, *Salsola zeyheri*, always contained inorganic phosphorus in the leaves, produced seed abundantly, and seed

formation was accompanied by a decline in phosphorus concentration in the leaves.

Phosphate absorption by plants in that region is limited apparently by the water shortage as well as by the small amounts of phosphorus present in the soil. In the case of *Digitaria eriantha*, growing with 22 inches of rain near Vryburg, abundant stalks and heads but no seeds were formed; with 19 inches, comparatively few heads appeared; and with 8½ inches only an occasional stem was found. At Ermelo, with 36 inches annual rainfall, another species of *Digitaria* produced abundant seeds, and the process was accompanied by decline in leaf phosphorus. The author does not state, however, whether soluble phosphorus was present in the leaves in this case. These results afford a strong indication that in the numerous species studied, the production of seeds depends largely upon whether there is more phosphorus available than is required for the organic cell constituents of the vegetative parts; and they also point to the inability of the plants to reutilize such organic phosphorus compounds, once they are built into the cell structure.

#### COMPARISON OF THE TWO FIRST-GENERATION CROSSES

##### TOTAL PHOSPHORUS

The total phosphorus data for the two strains may be compared in Figure 4. On untreated soil, the nonresponsive cross, 680, maintained a definitely higher concentration than cross 365 except at the last sampling. Computing from Table 6, the increases or decreases (—), in concentration of total phosphorus in No. 680 over No. 365 are, for the five periods, respectively, 25, 37, 16, 7, and —1 per cent. These figures evidently represent greater absorption of phosphorus rather than higher percentages due to less growth. Figure 1 is a typical representation of the two crosses growing on untreated soil shortly before the third sampling period. Photographs taken in three different years reveal the same situation. It will be observed that the average plant heights are about the same for the two crosses. Cross 365 is somewhat stockier than cross 680 and would probably be a little heavier. Unfortunately the data on plant weights and amounts of phosphorus per plant are incomplete. The available evidence would seem to indicate that cross 680 has greater ability to absorb phosphate from a limited supply. When, however, the supply is made adequate this cross fails to maintain a higher absorption rate. The increases or decreases (—) of total phosphorus, in No. 680 as compared to No. 365, computed as above, are for the five successive samplings 19, —2, 3, 3, —3 per cent. Under these conditions, with its greater growth (fig. 2) No. 365 has unquestionably taken up more phosphorus from the soil than has No. 680. Whether the greater growth is the result of more rapid phosphate absorption or whether the greater absorption is the result of the greater growth and more extensive root system of No. 365 can not be stated.

##### PHOSPHORUS FRACTIONS

Some of the results of the analyses of the two first-generation crosses are brought together for comparison in Figure 5. The supplying of abundant phosphate largely masks differences in the composition of the two strains, as may be seen in the close parallelism of the curves

for the superphosphate plots. (Fig. 5, C and D.) More pronounced differences are to be observed in the plants grown with limited available phosphate. The level of the nonlabile or protoplasmic phosphorus does not differ greatly in the two crosses, regardless of supply. However, with limited supply, No. 680, the nonresponsive cross, has a slightly higher level. It will be noted also that the two strains apparently reverse positions in this respect with the addition of abundant phosphate. It can not be stated that these small differences are outside the range of experimental error, but they are consistent throughout most of the period covered by the samplings. The chief differences between the two strains are to be found in the more labile fractions in plants which have been grown with inadequate phosphate. The nonresponsive cross, No. 680, contains a distinctly higher concentration of inorganic phosphorus during active vegetative growth. The difference gradually diminishes and disappears soon after pollination. (Fig. 5.) This is the only respect in which the two crosses deviate from each other appreciably in phosphorus composition. It can not be said that this difference is due to slower conversion of inorganic phosphorus to the various organic forms, for the variations in all three organic fractions, while they are too small to merit serious consideration, tend in the direction of higher concentrations in cross 680. This, in itself, is suggestive of a possible higher minimum requirement of phosphorus for the maintenance and functioning of the vegetative machinery in this cross as compared to the more responsive one.

#### SUMMARY AND CONCLUSIONS

It has been found in the case of open-pollinated yellow dent corn grown in the field that phytin is absent from all parts of the plant before pollination.

Within two weeks after pollination phytin appears in the developing seeds, but is not present in other parts of the plant. At this stage of growth there is an increasing gradient in total phosphorus concentration from the roots through the stalk and shank to the ear. These facts indicate a close association of the phytin-forming function with fertilization of the ovule, and the possibility is suggested that the activator of the zymogen of the enzyme responsible for phytin synthesis may be furnished by the pollen.

Phytin disappears from corn seeds at a fairly rapid rate during germination.

The phosphorus in the tops of two first-generation crosses, grown in the field in phosphorus-deficient soil, both with and without superphosphate fertilizer, was separated into one inorganic and three organic fractions at five stages of growth. Three of these stages were before pollination, one approximately at pollination, and one 11 days later. The two crosses differed in their response to phosphate fertilizers both in growth and in yield. The phosphorus fractions separated were: (1) Absolute alcohol extract (phospholipids); (2) inorganic; (3) organic, soluble in cold aqueous 2 per cent HCl; and (4) organic residue insoluble in this HCl (nucleic acids and nucleoproteins).

Two fractions, phospholipids and acid-insoluble organic phosphorus, remained fairly constant in percentage of the dry matter throughout the period of the experiment.



The other two fractions exhibited marked variations in concentration at successive samplings, the trend being toward a decrease in acid-soluble organic phosphorus throughout the duration of the experiment, and a progressive decrease in the inorganic fraction after the second sampling.

It is concluded as a result of this investigation that phosphorus translocated from vegetative parts of the plant into the ears consists only of these two labile fractions, namely, inorganic and acid-soluble organic compounds, with the possible exception of a breakdown of small amounts of phospholipids.

As a result of this work and a study of the literature, it is considered very doubtful whether phosphorus which has been laid down in essential organic constituents of plant cells can be transported and reutilized.

This investigation has not so far revealed a satisfactory explanation of the mechanism for the differential behavior of the two crosses studied with respect to phosphate fertilization.

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# THE BIOLOGY OF OPIUS MELLEUS GAHAN, A PARASITE OF THE BLUEBERRY MAGGOT<sup>1</sup>

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## INTRODUCTION

One of the interesting phases of the investigation of the blueberry maggot, *Rhagoletis pomonella* Walsh,<sup>2</sup> in eastern Maine (?)<sup>3</sup> proved to be a study of the biology of the parasite *Opius melleus* Gahan.<sup>4,5</sup> The study reported in this paper was conducted at Cherryfield, Me., during the years 1925 to 1929. The most intensive work was performed during the summer of 1929.

## HISTORY

*Opius melleus* was originally described in 1915 by Gahan (2, p. 73) from one female specimen found on Mount Washington, N. H., and was almost simultaneously described by Richmond (reported by Woods (10)) as *Biosteres rhagoletis* from 21 specimens reared from puparia of *Rhagoletis pomonella* from blueberries collected at Cherryfield, Me. In 1919 Gahan (4) cited *B. rhagoletis* as a synonym of *O. melleus*, and in the same year (3) he described two additional species, *O. richmondi* and *O. lectus*, both of which, he states, were swept from blueberry barrens in association with *O. melleus* and may also parasitize *R. pomonella*. Woods (10) was the first to rear *O. melleus* from puparia of the blueberry maggot, and he states that Severin reared it from the apple maggot. Good (5) described the method of oviposition into the apple maggot. Lathrop and Nickels (7) summarized the life history of *O. melleus*, gave tables indicating the percentage of parasitism of the blueberry maggot, and showed that this parasite could remain in the soil as long as four years and then successfully emerge as an adult.

During the present study *Opius melleus* proved to be the dominant parasite of the blueberry maggot in Washington County, Me., and was found in sufficient numbers to indicate that it is an important factor in the economy of its host. *O. richmondi*, reared in small numbers, was insignificant in comparison with *O. melleus*. No specimen of *O. lectus* was observed in the cages. If *O. lectus* parasitizes the blueberry maggot at all, it must be a minor species in eastern Maine.

## SOME HOST RELATIONSHIPS

*Rhagoletis pomonella* infests blueberries (*Vaccinium* spp.) and huckleberries (*Gaylussacia* spp.), and also apples (*Malus* spp.) and haws (*Viburnum* spp.). The insect is commonly termed "blueberry

<sup>1</sup> Received for publication Apr. 21, 1932; issued February, 1933.

<sup>2</sup> Order Diptera, family Tryptetidae.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 159.

<sup>4</sup> Order Hymenoptera, family Braconidae.

<sup>5</sup> The writers are indebted to A. B. Gahan for determinations of the species *Opius melleus* and *O. richmondi*.

maggot" or "apple maggot" according to the fruit with which it is associated. The two forms of the species appear to be ecologically distinct (6, 8), but no morphological basis for separation has been discovered,<sup>6</sup> although the apple maggot is distinctly larger than the blueberry maggot in every stage.

*Opius melleus* parasitizes both the apple maggot and the blueberry maggot. It is a significant fact that specimens of the parasite from the apple maggot are distinctly larger than specimens reared from the blueberry maggot. (Fig. 1.) Other than size, no structural difference affords a basis for separation of the species from the different hosts. Apparently *O. melleus* exhibits a tendency toward the development of two divergent strains parallel to the ecological forms of the host.

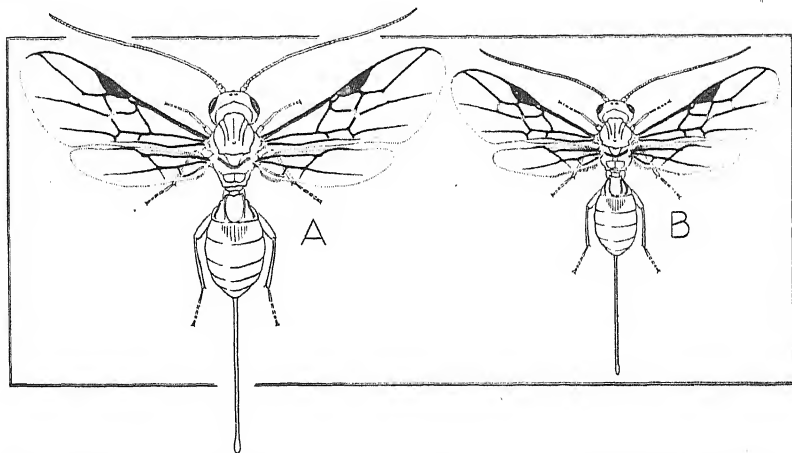


FIGURE 1.—*Opius melleus*; dorsal view of female with wings spread: A, From the apple maggot; B, from the blueberry maggot. X 8. Note the similarity of structure and the difference in size

#### LIFE HISTORY OF THE PARASITE IN RELATION TO THAT OF THE HOST

*Opius melleus* is well fitted to parasitize the blueberry maggot. The ovipositor of the female parasite is long enough to reach the host within the berry, and the life cycles of the two species coincide, to the advantage of the parasite. (Fig. 2.)

The flow of emergence of the adult parasites approximately parallels that of the flies, following it at an interval of 10 to 15 days. (Fig. 3.) Emergence of the females follows that of the males by 4 or 5 days. The earliest of the female parasites begin oviposition in 12 to 14 days after emergence, just as the maggots of the later instars appear in the berries in increasing numbers. The period of oviposition by the parasites coincides with the period of maximum abundance of maggots of the late second and third instars.

A portion of the host puparia remains in the soil for two years or more, thereby establishing a cycle of two years or longer. *Opius melleus* parallels this behavior, a portion of the population exhibiting a cycle of two or more years.

<sup>6</sup> Since this paper was written, C. H. Curran has described the blueberry maggot as a distinct species, *Rhagoletis mendax*, in American Museum Novitates No. 526, 1932. The validity of the species awaits confirmation by other dipterists.

The relationship existing between the blueberry maggot and its parasite, *Opius melleus*, is such as would be expected to occur between species in their native habitat, which has been occupied for an indefinitely long period under stable environmental conditions. Such conditions would permit the mutual adjustments which are exhibited by the maggot-parasite association.

### LIFE HISTORY OF OPIUS MELLEUS

The investigation of the life history of *Opius melleus* was conducted largely as a series of careful field studies, but in certain phases a combination of field and laboratory methods was employed.

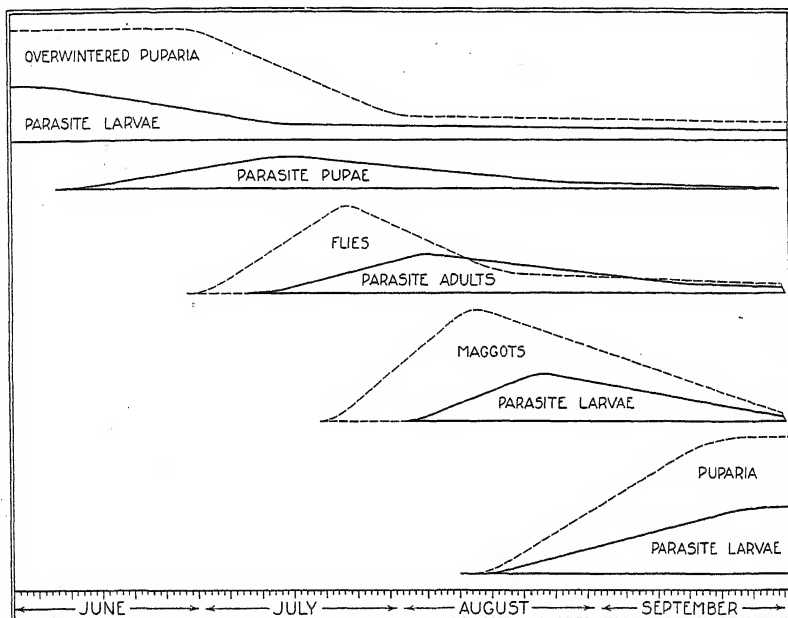


FIGURE 2.—Summary of life history of *Opius melleus* in relation to the blueberry maggot during an approximately normal season, Cherryfield, Me.

### EMERGENCE OF ADULTS

*Opius melleus* spends the winter as a full-grown larva within the puparium of its host just beneath the surface layers of the soil. (Fig. 2.) Pupation takes place within the enveloping puparium about 30 days before the adult parasite emerges.

When *Opius melleus* emerges from the puparium, it is apparently fully pigmented and the wings are fully expanded and hardened. The insect works its way upward through the soil by movements of the head, body, and appendages. Parasites that were observed in a glass-front observation box had much more difficulty in emerging than did the flies. The parasites showed distinct signs of fatigue after climbing upward through 2 inches of dry sand, and no parasites were observed to emerge from a depth of 4 inches. The flies emerged from this depth with comparative ease.

The difficulty experienced by the emerging parasites suggested that parasitized maggots might pupate at a more shallow depth in the soil than do nonparasitized individuals. A study of 1,694 puparia indicated that there is no significant correlation between the percentage of parasitism and the depth at which the puparia are normally found.

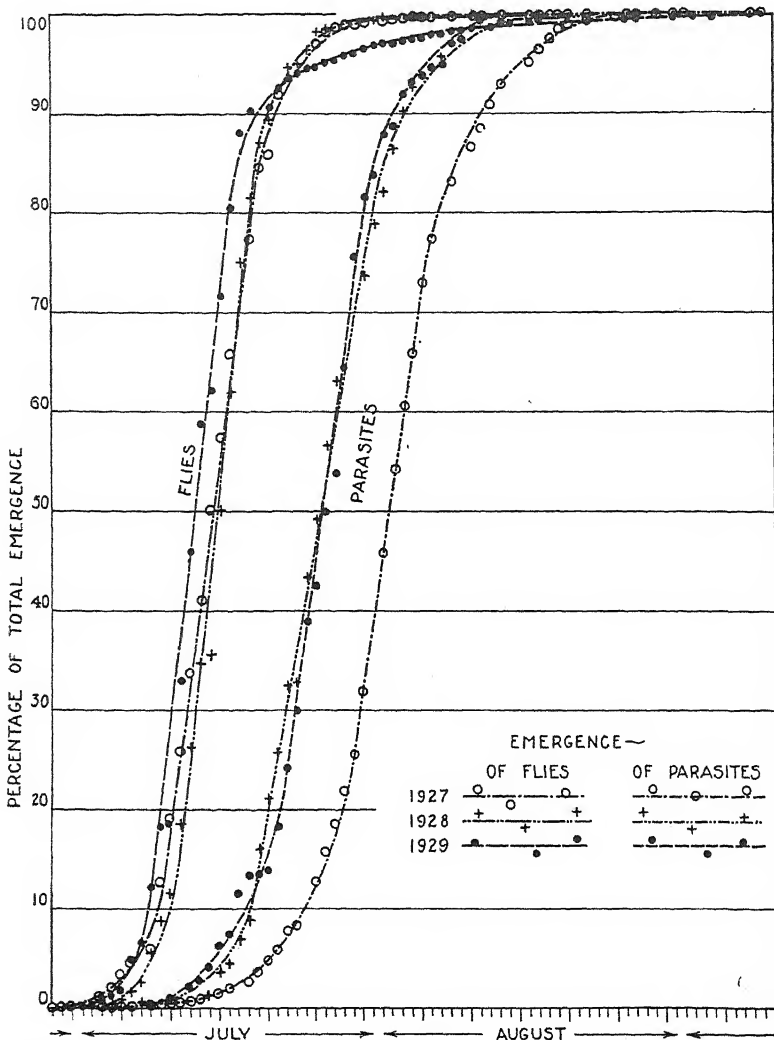


FIGURE 3.—Cumulative curves showing the emergence of adults of *Opus melleus* and its host during the seasons of 1927, 1928, and 1929

The fact that on an average 99 per cent or more of the puparia are found within  $1\frac{1}{2}$  inches of the surface of the soil makes it apparent that under field conditions the parasites have no serious difficulty in emerging from the soil.

The seasonal emergence of adults was studied by observations of the cages used in the studies of *Rhagoletis pomonella* by Lathrop and



Nickels (?). Blueberries heavily infested with maggots were placed on wire-screen trays over a series of plots during the late summer and autumn of each season. Each plot was 4 by 5 feet in size. The maggots dropped from the berries and entered the soil, where they pupated. During the following season each plot was covered by an emergence cage, and a careful record was made of the flies and parasites that emerged.

The emergence of flies and of parasites during the three seasons in which observations were made is best comprehended by a study of the accumulated emergence for each season. The records for the parasites are summarized in Table 1, and are illustrated graphically in Figure 3. It will be noted that the cumulative emergence curves have a characteristic form, depicting a slow rise early in the season, followed by a period of maximum emergence during which most of the adults appear, and a gradual cessation of emergence toward the end of the season.

TABLE 1.—Summary of emergence records of adults of *Opius melleus* for three years, Cherryfield, Me.

Year	Sex	Date of first emergence	Date of accumulated emergence in percentages indicated			Date of last emergence
			10	25	50	
1927	Male	July 3	July 21-22	July 27	July 30-Aug. 1	Sept. 17.
	Female	July 10	July 28-29	Aug. 1-2	Aug. 4-5	Sept. 14.
	Both		July 23-25	July 29	Aug. 1-2	
1928	Male	July 6	July 17-18	July 19-20	July 24-25	Aug. 24
	Female	July 15	July 20	July 23-24	July 28-29	Aug. 29.
	Both		July 18-19	July 21	July 25-26	
1929	Male	July 8	July 15-16	July 20-21	July 23-24	Aug. 11.
	Female	July 12	July 22-23	July 25-26	July 28-29	Aug. 12.
	Both		July 16-17	July 22-23	July 26	
			75	90	95	
1927	Male	July 3	Aug. 3	Aug. 8	Aug. 13	Sept. 17.
	Female	July 10	Aug. 8-10	Aug. 13-16	Aug. 18	Sept. 14.
	Both		Aug. 5-6	Aug. 12	Aug. 16	
1928	Male	July 6	July 28-30	Aug. 1-2	Aug. 4	Aug. 24.
	Female	July 15	Aug. 1-2	Aug. 6	Aug. 8-10	Aug. 29.
	Both		July 30-31	Aug. 3	Aug. 7	
1929	Male	July 8	July 27-28	July 30-31	Aug. 2-3	Aug. 11.
	Female	July 12	July 31-Aug. 1	Aug. 7-8	Aug. 9	Aug. 12.
	Both		July 29	Aug. 3	Aug. 7	

It is interesting to compare the emergence of the adult parasites with that of the flies. (Fig. 3.) Although the emergence of parasites is drawn out over a slightly longer period, the general form of the cumulative emergence curves is similar for both parasites and flies. The emergence curves of parasites during the seasons of 1928 and 1929 practically coincide, and might well be represented by a single line. The emergence of the parasites in 1927 occurred approximately seven days later than in 1928 and 1929, although the emergence of flies in 1927 coincided very well with that of 1928 and 1929. Probably the late emergence of parasites in 1927 was due to environmental influence of some kind, but the records of temperature and rainfall during the emergence periods do not afford any explanation.

## PROPORTION OF THE SEXES

During the early part of the emergence period the adults of *Opius melleus* which appear in the cages are largely males. Later the percentage of females increases, and toward the end of the period the females outnumber the males. In the total emergence of adults during the season, the males predominate. During the three seasons for which records are available the females constituted an average of 37.68 per cent of the adults captured in the cages. (Table 2.) It would seem that this tendency to produce excessive numbers of males might reduce the effectiveness of the parasite. The production of excessive numbers of males has been observed in other opiine parasites (9), and is probably due in part to parthenogenetic reproduction. Unmated females of *O. melleus* were observed to attempt oviposition, and it is probable that parthenogenesis occurs commonly in the field.

TABLE 2.—Proportions of the sexes of *Opius melleus* captured in emergence cages at Cherryfield, Me.

Year of emergence	Males		Females	
	Number		Number	Per cent
1927.....	2,491		1,549	38.34
1928.....	367		195	34.70
1929.....	216		144	40.00
Average.....				37.68

## CARRY-OVER IN THE SOIL FOR TWO OR MORE SEASONS

Like its host, the blueberry maggot, *Opius melleus* is capable of spending two or more seasons in the soil and then successfully emerging as an adult. From puparia formed in 1925 adults of *O. melleus* were observed to emerge during the summers of 1926, 1927, 1928, and 1929. Observations of emergence in eight cages over a period of three years are summarized in Table 3.

TABLE 3.—Carry-over of *Opius melleus* in the soil to the second and third season (host puparia formed in 1926)

Cage No.	1927		1928			1929			Total for the 3 years		
	Flies	Parasites	Flies	Parasites	Parasitism, as compared with 1927	Flies	Parasites	Parasitism, as compared with 1927	Flies	Parasites	Parasitism
	Number	Number	Number	Number	Per cent	Number	Number	Per cent	Number	Number	Per cent
A.....	3,937	462	79	30	6.49	2	0	0	4,018	492	10.91
B.....	4,676	65	67	11	16.92	2	1	1.54	4,745	77	1.60
2.....	2,564	42	46	8	19.05	2	0	0	2,612	50	1.88
3.....	1,401	82	76	40	48.78	9	6	7.32	1,486	128	7.93
5.....	475	20	35	7	35.00	5	0	0	515	27	4.98
8.....	2,691	687	273	146	21.25	23	12	1.75	2,987	845	22.05
9.....	2,365	997	358	320	32.10	59	14	1.40	2,782	1,331	32.36
11.....	2,498	566	532	191	33.75	22	8	1.41	3,052	765	20.04
Total...	20,607	2,921	1,466	753	25.78	124	41	1.40	22,197	3,715	14.34

\* Percentages based on totals.

## OCCURRENCE OF ADULTS IN THE FIELD

The occurrence of adult parasites in the field was studied in the summer of 1929 by making counts on unit areas, using the method developed by Lathrop and Nickels (7) in the study of the blueberry maggot. The plot under observation was divided into 20 count areas, each 50 by 100 feet. During the period of abundance of the adult parasites five men familiar with the parasites made counts twice a week, usually on consecutive days. Each man, provided with a light frame which inclosed an area 25 inches square (approximately 0.0001 acre), selected a place in the first count area and set his frame in position. When everyone was ready, a signal was given, and each man studied his unit area intently to observe any adult parasites present. At the end of two minutes, when another signal was given, the study of the area was discontinued and each man recorded the number and, if possible, the sex of the parasites observed. The frames were then moved to new places in the first count area, where another count was made. This process was repeated in each of the 20 count areas, and a total of 200 counts covering a combined area of 0.02 acre was made. The total number of parasites observed in the 200 counts, multiplied by 50, therefore represented the number of parasites per acre present on the plot.

The results of the counts are presented in Table 4 and shown in Figure 4. With the method described by Lathrop and Nickels (7), the data from the field counts were plotted simultaneously with data from the emergence cages to construct Figure 4. A study of these curves brings out some interesting facts regarding the fluctuations of the

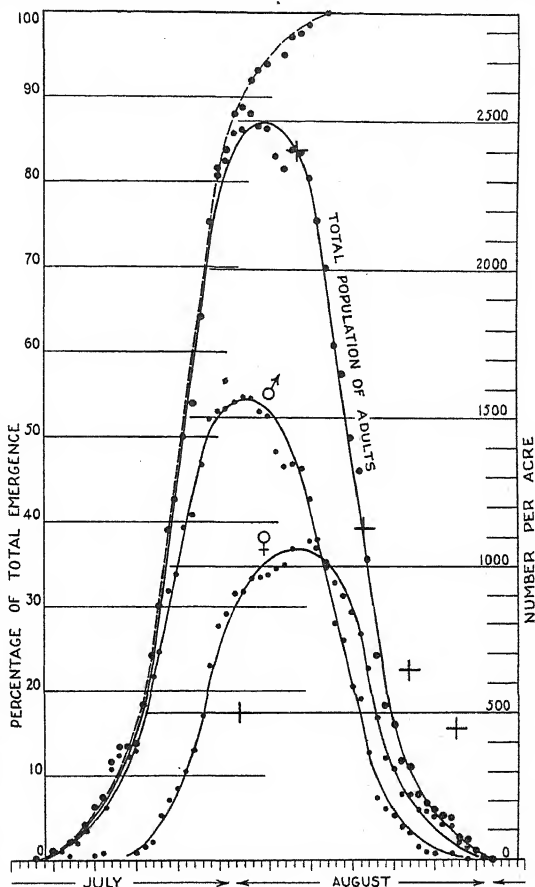


FIGURE 4.—Occurrence of adults of *Opius melleus* on experimental plot, Cherryfield, Me. Broken line indicates cumulative emergence of adults for the season of 1929. Upper curve shows the theoretical occurrence of adults throughout the season. The lower curves indicate the theoretical occurrence of adult males and females, respectively. The large crosses indicate the results of field counts of the total adult population on the dates indicated. All the population curves are based upon an average duration of life of 20 days. Note the agreement between the theoretical curves and the field counts

population of adult parasites. The numbers of parasites in the field rises rapidly as emergence proceeds. After the peak of occurrence is reached, the declining emergence and the increasing mortality result in a rapid decrease in the population. At the peak of occurrence there were present in the field more than 85 per cent of the total number of adults that emerged during the season. Theoretically, a total of approximately 2,800 or 2,900 *Opius melleus* adults per acre emerged on the area under observation. The males reached maximum numbers during the first few days in August, when approximately 1,500 individuals per acre were present. The maximum number of females occurred 7 to 10 days later with approximately 1,100 individuals per acre present in the field.

TABLE 4.—Field counts of adults of *Opius melleus*, 1929

Date	Adults of <i>Opius melleus</i>				Number per acre	
	Male	Female	Sex uncertain	Total	Each date	Average of pairs of counts
Aug. 1.....	8	3	0	11	550	}
Aug. 2.....	7	2	0	9	450	
Aug. 8.....	26	22	7	55	2,750	}
Aug. 9.....	21	16	4	41	2,050	
Aug. 16.....	15	12	0	27	1,350	}
Aug. 17.....	6	11	1	18	900	
Aug. 21.....	2	10	2	14	700	}
Aug. 23.....	5	7	0	12	600	
Aug. 27.....	4	5	1	10	500	}
Aug. 28.....	0	8	0	8	400	

## LONGEVITY OF ADULTS

Two methods were used in an effort to determine the duration of life of adults of *Opius melleus*: (1) The commonly used laboratory method of confining individuals in glass vials, where they were supplied with dilute sugar solution on pieces of blotting paper; and (2) the method of the simultaneous study of field counts and emergence records (7).

The data presented in Table 5 indicate that the average length of life of adults in the laboratory was 17.5 days. In this study there was no significant difference between the average length of life of males and of females. The simultaneous study of data from the field counts and the emergence records, as shown in Figure 4, indicates an average length of life of approximately 20 days. The field counts showed an apparent increase in the average length of life of the adults toward the end of the season.

TABLE 5.—Longevity of adults of *Opius melleus* in the laboratory, Cherryfield, Me., 1929

	Number of adults living number of days indicated <sup>a</sup>																												Total
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Males.....	1	0	0	1	2	2	3	3	5	6	7	6	11	6	8	20	17	18	22	7	5	3	2	6	1	0	1	163	
Females.....	0	1	0	2	3	2	6	4	3	5	7	3	8	9	9	9	7	9	12	3	6	6	2	1	2	1	2	122	
Total.....	1	1	0	3	5	4	9	7	8	11	14	9	19	15	17	29	24	27	34	10	11	9	4	7	3	1	3	285	

<sup>a</sup> Average duration of life was 17.8 days for males, 17.1 days for females, and 17.5 days for both.

## OVIPOSITION

Females of *Opisus melleus* confined in vials in the laboratory attempted oviposition within as short a time as five days after emergence. However, this does not necessarily mean that oviposition would take place so soon in the field. Data obtained from the emergence cages and from careful field observations led to the conclusion that the average preoviposition period in the field is considerably longer than the laboratory studies indicate.

During the season of 1929 adult females began emerging in numbers about July 20, as shown in Figure 4. Parasitism of the maggots did not develop in the field until about August 2, as shown in Figure 5 and Table 8. This apparently indicates that there is a preoviposition period of approximately 13 days. It may be argued that, since the eggs are deposited in maggots of the late second instar and the third instar, the parasites began oviposition as soon as host material was

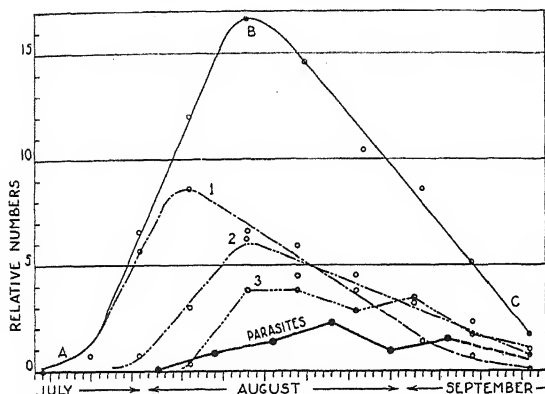


FIGURE 5.—Fluctuations in the population of immature stages of *Opisus melleus* in relation to the population of blueberry maggots on experimental plot, 1929. Curve A—B—C indicates the total maggot population in terms of units per 100 berries. Curves 1, 2, and 3 show the population of maggots in the first, second, and third instars, respectively. The heavy line indicates the relative number of *O. melleus* (immature stages). The data concerning the populations of the host are from Lathrop and Nickels (7)

available, and the delay was not due to a definite preoviposition period. However, the definite emergence of the female parasites 10 to 13 days prior to the development of suitable host larvae makes it appear probable that in the field there is a normal preoviposition period of approximately this duration.

The process of oviposition by the parasite was observed a few times in the field and rather commonly in the laboratory. The female examines the berry carefully with

her antennae. When she locates the maggot, she raises her body and brings the ovipositor down into position. (Fig. 6, A–D.) Then, by lowering the body, she thrusts the setae of the ovipositor deep into the berry. After a momentary pause, she removes the ovipositor from the berry and resumes her normal activity. The entire process of oviposition occupies 10 to 15 seconds.

In her search for host larvae in which to oviposit, the female parasite is apparently guided by the vibration produced by the movements of the maggots within the berries. The parasite could be induced to attempt oviposition into the empty skin of a blueberry by making movements beneath the blueberry skin with a needle or a pair of forceps to simulate the movements of a maggot. Maggots placed beneath the skin of a blueberry in a Petri dish stimulated oviposition, but the parasites made no attempt to oviposit directly into naked maggots crawling in the dish.

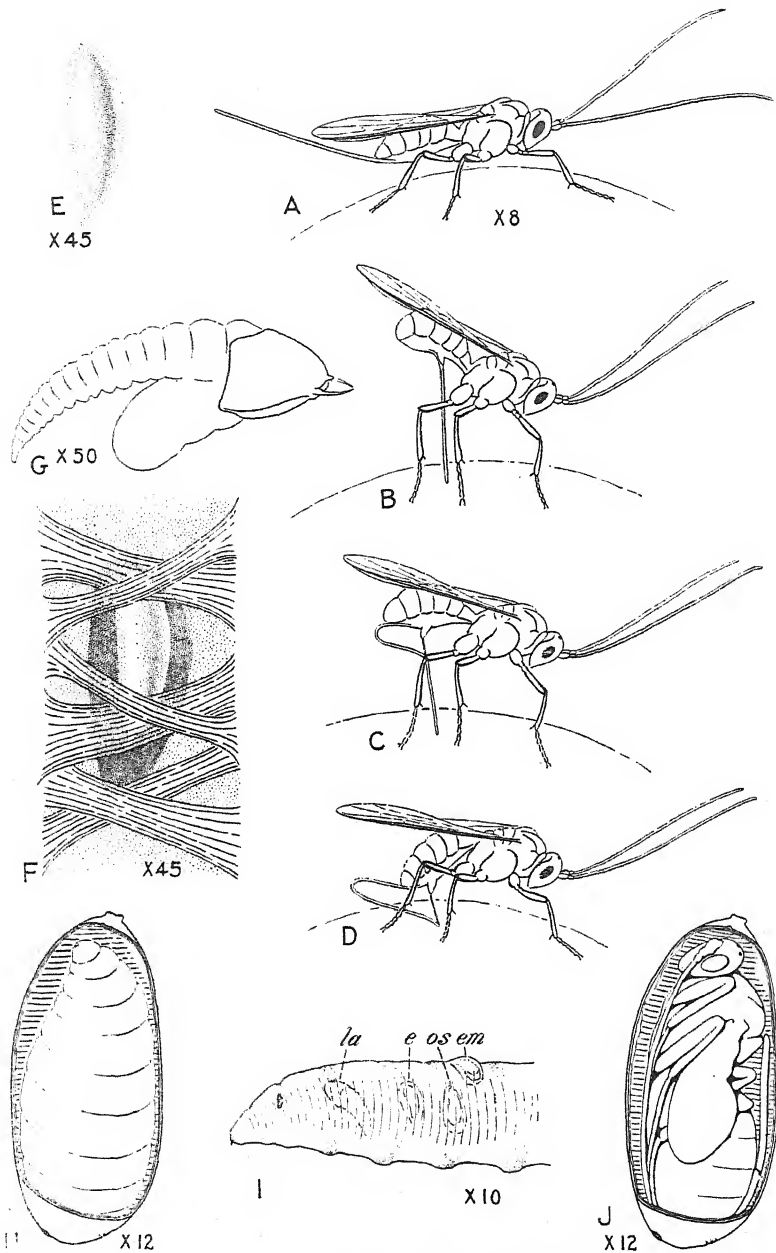


FIGURE 6.—A, *Opus melleus* on the surface of a blueberry, searching for a maggot in which to oviposit; B, C, D, three stages in the act of oviposition; E, egg of *O. melleus* removed from a blueberry maggot; F, egg of *O. melleus* viewed from the inner surface of the body wall of the blueberry puparium, showing the position of the egg between the epidermis and the muscle fibers; the egg is surrounded by a pellucid area or "blister," which shows dark in the illustration; G, newly hatched larva of *O. melleus*, removed from the body of a blueberry puparium; H, mature puparium of *O. melleus*, from which a portion of the wall has been removed; I, anterior portion of a blueberry puparium, showing a recently deposited egg (e), an egg containing a well-developed embryo (em), a newly hatched larva (la), and an ovipositor scar (os) of the parasite, *O. melleus*; J, pupa of *O. melleus* within the puparium of the blueberry puparium

The egg may be deposited within the maggot in a dorsal, ventral, or lateral position and anywhere from the anterior to the posterior extremities. Usually the egg is found just beneath the skin of the maggot, between the muscle fibers and the outer cuticle. (Fig. 6, E, F.) It is possible that the parasite eggs may be placed in other parts of the maggot also, where they are more difficult to see, and therefore where they are less frequently discovered. One egg was observed loose in the body cavity of a maggot, and was dissected out. This egg may have been placed just beneath the skin of the maggot, and accidentally dislodged and set free in the body cavity.

The newly deposited egg is inconspicuous, but as the embryo develops the egg increases in size, and usually produces a somewhat more readily observed "blister" on the skin of the maggot. (Fig. 6, I.) The point of entrance of the ovipositor is frequently marked by a darkened point on the integument of the maggot. The darkened spot could not always be discovered, however, and it could not be determined that the egg was held in place by any cementing material. Apparently the maggots were frequently pierced by the parasite without an egg being deposited. At least, what appeared to be oviposition scars were commonly observed where no egg could be discovered. That the ovipositor may sometimes be thrust entirely through the maggot is indicated by the fact that oviposition scars are sometimes found in pairs on opposite sides or nearly opposite sides of the maggot.

Oviposition scars in the third-instar maggots may persist in the puparia, and apparently the scars sometimes provide points of entry of bacterial or fungous organisms, which attack the tissues within.

It is not uncommon to find two or more eggs in one maggot. A maggot was once observed to contain four first-instar parasite larvae. Apparently when more than one larva is present in a puparium there is a struggle for survival. In one instance a puparium was dissected and found to contain two battling parasite larvae. Never has more than one mature larva been observed in a puparium.

#### INCUBATION PERIOD

The incubation period of the egg of *Opius melleus* was not satisfactorily determined in this study. Willard (9) found that the eggs of *O. fletcheri* in Hawaii hatched in 37 to 40 hours after oviposition. As might be expected, the egg of *O. melleus* in Maine required a much longer time. Limited observations indicate that the egg of *O. melleus* requires an incubation period of from three to six days. Some eggs deposited in the nearly mature maggots were observed to hatch within the puparia.

#### LARVAL DEVELOPMENT

The first-instar larva (fig. 6, G) is actively motile, and swims freely within the body cavity of the host. In this stage the larva is provided with a pair of long, sharply pointed jaws. The parasite larva has never been observed to transform to the second instar until after the formation of the host puparium. Then the larval development of the parasite usually proceeds rapidly. In most cases under observation the parasite larvae attained the fourth instar within 10 days after the puparium was formed. Larval development may be delayed,

however, one puparium being observed to contain two living parasite larvae in the first instar at least 4 weeks after the formation of the puparium. The larval transformations are complete, nevertheless, before freezing temperature occurs.

The parasite larva in the second, third, and fourth instars is inactive, and is without conspicuous appendages. The host tissues appear to undergo normal histolysis after the formation of the puparium. The pupal structures are not formed, however; instead, the substance of the host is ingested by the growing parasite larva. The mature parasite larva almost completely fills the puparium. (Fig. 6, H.) Usually the walls of the puparium, when moist, are translucent, and the presence of a parasite larva may be detected by external examination of the puparium. The exuviae of the preceding instars are pressed against the wall of the puparium by the larva, and the old head capsule of the first instar may often be seen through the wall of the puparium containing a mature larva of *Opius melleus*. It is not uncommon to find two or more head capsules of first-instar larvae within a single puparium (in a few cases four head capsules were found), an indication of the competition of several larvae and the survival of but one.

#### THE IMPORTANCE OF OPIUS MELLEUS

The quantitative study of the relation of *Opius melleus* to its host is as difficult as it is interesting. The problem consists of two phases, the determination of the percentage of parasitism and the evaluation of the effect of the parasite upon the economy of its host.

#### THE DETERMINATION OF PERCENTAGE OF PARASITISM

In estimating the degree of parasitism the objective is to determine the percentage of blueberry maggots attacked by *Opius melleus* throughout the season, thereby indicating the proportion of the total population of maggots destroyed by the parasite. In view of the life habits of the parasite, and the single-generation cycle of both parasite and host, the ideal method of estimating the percentage of parasitism would be the examination of a representative sample of puparia taken from the soil of the area being studied. The sample of puparia should be obtained during the dormant period when both parasite and host are in a hibernating condition. Under normal conditions in the field, however, large quantities of soil must be examined to obtain a very few puparia of the blueberry maggot, even on areas supporting a large maggot population. It was therefore found impracticable to obtain a representative sample of puparia, and it was necessary to resort to other methods for making quantitative estimates of parasitism.

Two methods were used to determine the empirical percentage of parasitism of maggots from samples of blueberries: (1) Placing the berries over pupation plots, and determining the ratio of parasites to flies captured in emergence cages placed over the plots during the following season; and (2) examining puparia obtained from samples of blueberries. The results of two series of determinations are summarized in Tables 6 and 7.



TABLE 6.—*Parasitism of the blueberry maggot by Opius melleus*

[Records from emergence cages, 1927]

Cage No.	Flies	Parasites	Parasitism	Cage No.	Flies	Parasites	Parasitism
	<i>Number</i>	<i>Number</i>	<i>Per cent</i>		<i>Number</i>	<i>Number</i>	<i>Per cent</i>
A.....	3,937	462	10.50	8.....	2,691	687	20.34
B.....	4,676	65	1.37	9.....	2,365	997	29.66
1.....	1,192	17	1.41	10.....	1,101	302	21.53
2.....	2,564	42	1.61	11.....	2,498	566	18.47
3.....	1,401	82	5.53	12.....	2,447	142	5.49
4.....	1,085	90	7.66	13.....	1,567	297	15.93
5.....	475	20	4.04	14.....	405	64	13.65
6.....	1,738	169	8.86	Total.....	30,853	4,040	" 11.58
7.....	711	38	5.07				

a Percentage based on totals.

TABLE 7.—*Parasitism of the blueberry maggot by Opius melleus*

[Determinations made by examination of puparia]

Lot No.	Date berries were picked	Puparia examined	Puparia parasitized		Source of berries
	1927	<i>Number</i>	<i>Number</i>	<i>Per cent</i>	
1.....	Aug. 16.....	340	133	39.12	{ A homogeneous sample picked on poorly tended land. A high percentage of the berries was infested with maggots.
2.....	do.....	224	85	37.95	
3.....	Aug. 20-24.....	118	24	20.34	Accumulated as left-overs of samples from check plots.
4.....	do.....	77	19	24.68	Accumulated as left-overs from samples from plots dusted with calcium arsenate.
5.....	Aug. 25.....	188	39	20.75	Picked on brush-free portions of check plot.
6.....	do.....	195	56	28.72	Picked on bushy portion of check plot.
7.....	do.....	61	2	3.28	Picked on brush-free portions of plot dusted with calcium arsenate.
8.....	do.....	115	23	20.00	Picked on bushy portions of check plot.
9.....	Aug. 26.....	196	93	47.45	{ A homogeneous sample picked on poorly tended land. A high percentage of the berries was infested with maggots.
10.....	do.....	257	127	49.42	

At first thought it would seem that a determination of the percentage of parasitism of a series of samples, such as that shown in Tables 6 and 7, would give a reasonably satisfactory basis for estimating the efficiency of *Opius melleus*, especially if the samples are large enough to yield reliable data, are numerous enough to indicate the range of variation, and extend fairly well over the season. A more thorough consideration, however, reveals several reasons why such a series in itself can not indicate, even approximately, the true numerical relation of *O. melleus* to its host.

Each of the determinations of parasitism shown in Tables 6 and 7 indicates the percentage of the entire population of maggots of all stages that were parasitized at the time the sample of berries was picked. As only the maggots of the late second and third instars are subject to parasitism, however, it is evident that a determination of the percentage of parasitism of the entire population of maggots does not give a fair indication of the effective parasitism. Willard (9), in working with *Opius fletcheri* as a parasite of the melon fly in Hawaii, observed a similar situation, and overcame the difficulty by basing estimates of parasitism only upon maggots which emerged from the cucumbers—within which the hosts fed—during the first two to four days after collection. Such a method can not be em-

ployed in the case of *O. melleus*, because the blueberry maggot remains in the berries for an abnormally long time if the berries are picked and placed where they are not subject to normal weathering. Even were it possible to base the estimate on the percentage of parasitism of susceptible maggots only, a series of samples picked from various localities and at different dates would give little indication of the efficacy of *O. melleus*. The reason for this is that each sample (Tables 6, 7, and 8) represents a cross section of the maggot-parasite association considered as a static population, and shows the percentage of parasitism existing in this supposedly static population at the moment that the sample was taken. In reality the maggot-parasite population is by no means in a static condition. The condition might more properly be regarded as a moving stream terminating in a reservoir, or rather as a combination of two streams, one of maggots and the other of parasites, each flowing at a different rate. With such a conception in mind, it is clear that a cross section of such a composite stream, or even a series of cross sections, will not indicate the relative percentage of maggots and parasites in the reservoir in which the stream terminates unless the relative rates of flow of maggots and of parasites are considered.

TABLE 8.—Seasonal study of parasitism of the blueberry maggot by *Opius melleus* on experimental plot, Cherryfield, Me., 1929

Date	Parasitism of puparia from field samples	Total maggot population per 100 berries <sup>a</sup>	Relative numbers of parasites present throughout season	Maggots present per 100 berries <sup>b</sup>	Maggots parasitized
	<i>Per cent</i>	<i>Number</i>		<i>Number</i>	<i>Per cent</i>
Aug. 2.....	0.50	10.8	0.05	8.2	0.61
Aug. 9.....	5.50	16.2	.89	14.5	6.14
Aug. 16.....	8.36	16.8	1.41	16.2	8.70
Aug. 23.....	16.98	13.5	2.29	13.2	17.35
Aug. 30.....	9.45	10.1	.96	9.8	9.80
Sept. 6.....	22.91	6.6	1.51	6.5	23.23

<sup>a</sup> Including eggs and maggots.

<sup>b</sup> Not including eggs.

Clausen and King (1, p. 9-10) were confronted by a somewhat parallel problem in their work with *Centeter cinerea* Ald., a parasite of *Popillia japonica* Newm. These authors showed that the host was killed within six days after the parasite egg was deposited; therefore, practically all beetles bearing eggs on any given date are dead within six days from that time, and the parasitized beetles in the field at a later date represent an additional percentage of the total.

To illustrate \* \* \*. On July 14, 35 per cent bore eggs, these being replaced on the 20th by 45 per cent of the remainder, the latter in turn being replaced by 48.5 per cent of those remaining on the 26th. Thus, theoretically, the parasitism effected was 35 plus 29 plus 17 per cent, successively, during the period of abundance, totaling approximately 81 per cent of the entire infestation.

In the case of *Opius melleus*, the total parasitism can not be deduced by a summation of this kind. *O. melleus* does not immediately destroy its host, and the length of time that the parasite spends in the host larva is variable.

An interesting view of the problem is presented by the data in Table 8, shown graphically in Figure 5. A large sample of blueberries was collected from the experimental plot once a week throughout the season. The berries were placed over sand boxes. In the autumn, after pupation was complete, the puparia were collected and examined to determine the percentage of parasitism in each of the weekly samples. In considering these data it should be borne in mind that the parasite is present during only a portion of the life of the maggot. Therefore, the effective parasitism is somewhat higher than the ratio of parasites to total maggots in the population. If the relative duration of life of the maggot and of the parasite egg and larva combined were known, the total parasitism for the season might be determined from the data summarized in Figure 5. For example, if the parasite eggs were laid only in the newly hatched maggots, then the parasite would be present throughout the life of the maggot, and the curve showing the relative number of parasites must coincide throughout the season with the curve (A-B-C) of the total maggot population to indicate 100 per cent parasitism. If the duration of life of the parasite within the maggot were equal to that of the third instar of the maggot, then the parasite curve in Figure 5 should coincide with curve 3 throughout the season to indicate 100 per cent parasitism. Actually, the parasite eggs are deposited at any time after the maggot approaches the third instar. Many eggs are deposited in maggots of the second instar; others are deposited in full-grown maggots on the point of leaving the berries. It seems probable that the average duration of life of the parasite within the maggot is nearly equal to the duration of the third instar of the host. It would seem, therefore, that a fairly accurate estimate of the total seasonal parasitism of the blueberry maggot by *Opius melleus* on the plot under observation might be arrived at by a determination of the relative areas inclosed by curve 3 and by the "parasite" curve in Figure 5. A comparison of the areas of the two figures by means of a planimeter indicated a ratio of 1 to 0.4919. There was, therefore, a total seasonal parasitism of 49.19 per cent on the experimental area under observation.

#### EFFECT OF OPIUS MELLEUS UPON THE ECONOMY OF ITS HOST

If the determination of the total parasitism by *Opius melleus* is difficult, the estimation of the effect of the parasite upon its host is much more so, and in the present state of knowledge must be little more than careful conjecture.

Discussing the importance of *Opius fletcheri*, Willard (9, p. 434) states: "While this parasite alone will never exercise a complete control over the melon fly in Hawaii, it has already proved of much value by decreasing the numbers of this pest considerably." Data given by Willard show that the highest parasitism obtained in his study was 29 per cent of the susceptible larvae (melon-fly larvae emerging from cucumbers within two to four days after picking). The average parasitism was 18.1 per cent.

The data presented in the present study show parasitism by *Opius melleus* as high as 49 per cent. The average parasitism in Table 6 is 11.58 per cent and in Table 7 is 29.17 per cent. These estimates are based on the entire maggot population of the berries at the time the

samples were picked, and the total parasitism for the season has been shown to be considerably higher than estimates based upon the total maggot population of isolated samples.

It is evident that the percentage of parasitism of the blueberry maggot by *Opius melleus* is as great as that which occurs in the case of some parasites which are considered important factors in reducing the population of imported insect pests. The percentage of parasitism alone, however, does not necessarily indicate the potential value of the parasite, for the effect of the parasite upon the abundance of the host is dependent upon a number of other factors, such as comparative rates of reproduction and ecological competition.

*Opius melleus* and its host, the blueberry maggot, are both native to eastern Maine. The association between the two species has no doubt existed sufficiently long for the complete establishment of mutual adjustments between the economy of the parasite and that of its host. It is not to be supposed, therefore, that *O. melleus* is actively reducing the population of the blueberry maggot, or that it will ever exert such an effect unless there should occur some marked change in the environment of the species. It seems clearly indicated, however, that this parasite is an important ecological factor in the balance which apparently exists in the blueberry-maggot association.

#### SUMMARY

In connection with an investigation of the blueberry maggot in eastern Maine during the years 1925 to 1929, a study was made of the biology of the parasite *Opius melleus* Gahan.

*Opius melleus* is well fitted to parasitize the blueberry maggot. The ovipositor of the female parasite is long enough to reach the host within the berry, and the life cycles of the two species parallel each other, to the advantage of the parasite.

The full-grown larva of the parasite spends the winter within the puparium of the host just beneath the surface layers of the soil. Pupa-tion takes place within the puparium about 30 days before the adult parasite emerges. Adults begin to emerge during the first two weeks in July, and in normal seasons emergence is almost complete by the middle of August. Oviposition begins early in August and continues during the period of abundance of suitable host larvae.

During the three seasons for which records are available the females constituted an average of 37.68 per cent of the adults captured in emergence cages.

Like its host, the blueberry maggot, *Opius melleus* is capable of remaining in the soil for two or more seasons and then successfully emerging as an adult.

Fluctuations of the population of adult parasites were studied by counts of unit areas in the field. The males reached maximum numbers during the first few days in August, when approximately 1,500 individuals per acre were present. The maximum number of females occurred 7 to 10 days later, with approximately 1,100 individuals per acre.

Laboratory studies indicated that the average length of life of the adult parasites was 17.5 days, with no significant difference in the longevity of males and females. The simultaneous study of data

from field counts and the emergence records indicates an average length of life of approximately 20 days.

The female parasite deposits the egg directly into the body of the maggot. The egg hatches within three to six days. The parasite larva swims freely in the body cavity of the maggot, without injury to the host, until the host puparium is formed. Parasite larvae may be found in the maggots from early August until frost occurs—usually in late September or early October.

After the formation of the puparium by the host, the parasite larva undergoes rapid development, and the larval transformations are complete before freezing temperatures occur.

Records of adults captured in emergence cages indicated parasitism of the blueberry maggot by *Opius melleus* ranging from 1.37 to 29.66 per cent. Examinations of puparia indicated parasitism ranging from 3.28 to 49.42 per cent. A thorough study of the problem shows that the determination of the percentage of parasitism of isolated samples does not indicate the effective seasonal parasitism by *O. melleus*. The most accurate estimate of the total seasonal parasitism was made by plotting the seasonal populations of maggots of the third instar and of parasites, and comparing the areas inclosed by the respective curves. (Fig. 5.)

The percentage of parasitism alone, however, does not necessarily indicate the potential value of the parasite in reducing the population of the blueberry maggot, for a number of other factors, such as relative rates of reproduction and ecological competition, must be taken into consideration. Inasmuch as both the parasite and its host are native to eastern Maine, their association has no doubt existed sufficiently long for the establishment of mutual adjustments in their economy. *Opius melleus* is not actively reducing the population of the blueberry maggot, and it will probably never have this effect unless some marked change in the environment of the species should occur. It seems clearly indicated, however, that this parasite is an important ecological factor in the balance which apparently exists in the blueberry-maggot association.

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# BASE-EXCHANGE MODIFICATIONS OF A LEONARDTOWN SILT LOAM UNDER FERTILIZER AND CROP CONTROL<sup>1</sup>

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## INTRODUCTION

The extensive examination of the colloid of Leonardtown silt loam, the dominant type of the Leonardtown soil series, by Holmes<sup>2</sup> revealed the fact that the colloid of this soil is remarkably constant in composition so far as the main analytical constituents are concerned, but that the basic and acidic components are rather variable. The Maryland Agricultural Experiment Station has for the last 12 years been conducting fertilizer treatment tests on a series of plots of Leonardtown silt loam situated about three-quarters of a mile east of La Plata, Charles County, Md. This area is extremely uniform in topography,

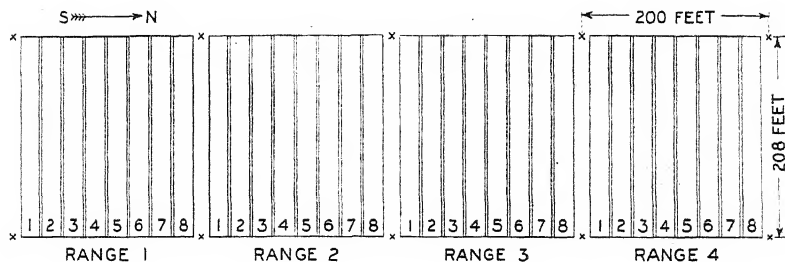


FIGURE 1.—Ranges and plots at the Maryland Agricultural Experiment Station, soil-fertility investigations, La Plata field. Only plots 1, 2, and 4 in each range were studied. The alleys between the ranges are 18 feet wide; the plots, 21 feet wide with 2-foot alleys between them. X indicates iron stakes set in concrete

and when the experiments were begun was very unproductive. The treatments given certain plots resulted in a marked alteration of crop yields. This area, therefore, seemed to offer special opportunity for a study of the base-exchange relationships between the untreated soil and the soil with improved fertility.<sup>3</sup> It was hoped that such a study might reveal the changes in chemical composition of the colloid which were responsible for the marked alteration of crop yields effected by the soil treatment.

## DESCRIPTION OF AREA AND SOIL TREATMENT

The soil under examination is a very level and apparently uniform area. It was divided into ranges and plots as indicated in Figure 1. In each range the examination was limited to the most productive

<sup>1</sup> Received for publication Apr. 25, 1932; issued February, 1933.

<sup>2</sup> HOLMES, R. S. VARIATIONS OF THE COLLOIDAL MATERIAL IN TYPICAL AREAS OF THE LEONARDTOWN SILT LOAM SOIL. Jour. Agr. Research 36: 459-470. 1928.

<sup>3</sup> The data concerning the plots were placed at the writer's disposal through the kindness of Prof. J. E. Metzger and E. H. Schmidt, of the agronomy department of the University of Maryland.

plots numbered 1 and 2, and to the check plots numbered 4. In October, 1920, preliminary to starting the work, all the plots received a presumably uniform addition of limestone at the rate of 2 tons an acre. The operation of the plots began in the autumn of 1920. The whole series of plots was cultivated under the same 4-year rotation—corn, soybeans, wheat, and grass. The plots, when in corn, were given a cover-crop treatment of rye, which furnished green manure for the soybeans. Plot 1 in each range received, previous to corn planting, 5 tons of manure an acre, together with 1,000 pounds of ground rock phosphate; each wheat crop received 5 tons of manure only. Plot 2 in each range received 10 tons of manure an acre for corn, and 400 pounds of superphosphate for each wheat crop. At the time of sampling, October, 1930, the total manure that had been applied to plot 1 in each range was 26.25 tons an acre; and to plot 2, 25 tons an acre. Plots 1 had received 2,500 pounds of rock phosphate and plots 2, 1,200 pounds of superphosphate an acre. The effect of this treatment on the crop yield is indicated in Table 1 by the average yields for the 4-year period 1926–1929 and the yields for 1931.

TABLE 1.—Yields on La Plata plots

Year	Plot No.	Wheat	Corn	Soy-beans	Hay	Year	Plot No.	Wheat	Corn	Soy-beans	Hay
		<i>Bush.</i>	<i>Bush.</i>	<i>Bush.</i>	<i>Tons</i>			<i>Bush.</i>	<i>Bush.</i>	<i>Tons</i> <sup>a</sup>	<i>Tons</i>
4-year period 1926–1929...	1-----	17.51	36.61	14.03	2.28	1931-----	1-----	30.2	59.1	1.41	1.00
	2-----	14.15	36.72	14.19	1.34		2-----	26.7	47.7	1.18	.65
	4-----	8.16	15.22	5.75	.83		4-----	11.0	27.9	.80	.30

<sup>a</sup> The soybean crop for 1931 was harvested as hay.

Although the growing season of 1931 was especially favorable for certain crops, and the actual yields of wheat and corn greatly exceeded the 4-year average, the difference between the plots which had been fertilized and the check plots is equally well marked.

#### COLLECTION AND EXAMINATION OF SAMPLES

By means of a King sampling tube 20 samples were taken along the longitudinal center of each plot to the mean plow depth, about 5 inches. These samples were thoroughly mixed, and the composite material examined. Range 2 was in corn, and some difficulty was experienced in obtaining subsamples of uniform depth, because of surface irregularities. Range 3 was in soybeans, and the surface was in a very pulverulent condition, which also rendered accurate sampling difficult. Range 1, in clover, and range 4, in wheat, were more level and compact, and the samples from these ranges doubtless represent the mean soil values better than those from ranges 2 and 3. The samples were subjected to mechanical analysis by the pipette method.<sup>4</sup> The data so obtained are given in Table 2.

<sup>4</sup> OLMSTEAD, L. B., ALEXANDER, L. T., and MIDDLETON, H. E. APIPETTE METHOD OF MECHANICAL ANALYSIS OF SOILS BASED ON IMPROVED DISPERSION PROCEDURE. U. S. Dept. Agr. Tech. Bul. 170, 23 p., illus, 1930.



TABLE 2.—*Mechanical analyses of composites from different soil plots*

Range No.	Plot No.	Fine gravel, 2-1 mm	Coarse sand, 1-0.5 mm	Medium sand, 0.5-0.25 mm	Fine sand, 0.25-0.1 mm	Very fine sand, 0.1-0.05 mm	Silt, 0.05-0.005 mm	Clay, 0.005-0 mm	Inorganic colloid, 0.002-0 mm	Loss by H <sub>2</sub> O <sub>2</sub> treatment
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	1.....	1.1	4.5	7.1	9.6	5.4	51.4	20.9	12.0	1.8
	2.....	.8	4.4	6.9	8.8	5.6	52.6	20.9	11.9	2.6
	3.....	.6	4.5	7.1	8.8	5.2	54.4	19.4	11.1	1.5
	4.....	.7	4.3	8.0	10.5	6.0	52.8	17.7	10.6	1.8
2	1.....	.8	4.2	8.5	11.0	5.6	50.8	19.1	11.7	1.9
	2.....	.4	3.7	8.4	11.3	5.9	49.1	20.6	13.2	1.6
	3.....	1.0	3.9	7.7	10.5	6.0	51.0	19.9	12.5	1.8
	4.....	.7	4.6	8.3	11.3	6.4	50.1	18.7	11.6	1.4
3	1.....	.7	4.8	8.3	11.3	6.8	50.0	18.1	11.3	1.2
	2.....	.5	3.9	7.5	10.0	6.5	52.6	19.1	11.2	2.0
	3.....	1.1	3.9	6.9	9.6	6.1	51.6	20.8	12.3	1.8
	4.....	.6	5.0	6.7	9.1	6.5	53.0	18.1	10.8	1.4

The samples were not subjected to complete chemical analysis, nor were the colloids analyzed because of the known character of the material.<sup>5</sup>

Two hundred grams of each sample was digested with 2 liters of 0.05 normal hydrochloric acid for 18 hours and then filtered and washed by means of a Pasteur-Chamberland filter. The filtrate was evaporated and analyzed. This procedure may be expected, as shown by Mattson,<sup>6</sup> to extract essentially the same base-exchange and acid-exchange materials as would be obtained by electrodialysis. The results obtained, expressed as milliequivalents per 100 g<sup>7</sup> of dry soil, are given in Table 3.

TABLE 3.—*Constituents of soil soluble in N/20 hydrochloric acid*

[Results expressed in milliequivalents per 100 g of air-dry soil]

Range No.	Plot No.	Ca	Mg	K	Na	Mn	Al	Fe	Si	P <sub>2</sub> O <sub>5</sub>
1	1.....	3.36	0.30	0.17	0.09	0.68	6.66	0.34	0.50	0.22
	2.....	2.28	.27	.13	.06	.82	4.65	.35	.53	.05
	3.....	2.22	.21	.07	.07	.56	4.26	.30	.60	.02
	4.....	4.98	.47	.26	.27	.51	13.30	.75	1.42	.20
2	1.....	5.78	.63	.22	.28	.59	11.30	.64	1.92	.06
	2.....	2.67	.33	.14	.38	.51	11.60	.45	1.52	.02
	3.....	5.15	.63	.23	.23	.73	16.30	.53	2.12	.21
	4.....	3.06	.58	.22	.24	.56	11.88	.41	1.82	.06
3	1.....	6.32	.80	.15	.24	.39	9.65	.37	1.76	.02
	2.....	4.54	.47	.20	.08	.93	6.88	.37	.80	.28
	3.....	2.73	.35	.15	.07	.65	5.29	.27	.60	.06
	4.....	2.00	.29	.07	.08	.45	4.18	.22	.60	.03
Average of plots numbered 1.....		4.51	.47	.22	.17	.71	10.78	.50	1.21	.23
Average of plots numbered 2.....		3.46	.46	.18	.16	.65	8.28	.42	1.22	.06
Average of plots numbered 4.....		3.30	.41	.11	.19	.48	7.42	.33	1.12	.02

The base-exchange components were also determined by leaching 100 g of each of the samples with 1 liter of normal ammonium acetate solution of pH 7. The procedure followed was that of Schollenberger

<sup>5</sup> HOLMES, R. S. Op. cit.<sup>6</sup> MATTSON, S. ELECTRODIALYSIS OF THE COLLOIDAL SOIL MATERIAL AND THE EXCHANGEABLE BASES. Jour. Agr. Research 33: 553-567, illus. 1926.<sup>7</sup> g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

and Dreibelbis.<sup>5</sup> The leachate was evaporated to dryness and analyzed. The results obtained are given in Table 4.

TABLE 4.—*Constituents of soil soluble in normal ammonium acetate solution*

[Results expressed in milliequivalents per 100 g. of air-dry soil]

Range No.	Plot No.	Ca	Mg	K	Na	Mn	Al	Si
1	1.....	2.87	0.53	0.16	0.19	0.07	0.52	0.10
	2.....	2.95	.45	.15	.19	.06	.48	.13
	3.....	3.08	.43	.11	.15	.06	.42	.04
	4.....	4.00	.54	.20	.40	.19	.60	.17
2	1.....	5.35	.72	.12	.38	.17	.21	.43
	2.....	2.86	.37	.10	.46	.17	.62	.24
	3.....	4.10	.50	.14	.43	.06	.56	.25
	4.....	3.50	.48	.16	.43	.14	.60	.32
3	1.....	6.83	.47	.06	.38	.06	.38	.30
	2.....	6.28	.58	.21	.16	.07	.50	.09
	3.....	2.97	.62	.15	.17	.07	.41	.16
	4.....	2.21	.35	.10	.18	.07	.50	.36
Average of plots numbered 1.....		4.31	.54	.18	.29	.10	.54	.15
Average of plots numbered 2.....		3.69	.57	.14	.29	.11	.42	.26
Average of plots numbered 4.....		3.74	.40	.09	.29	.09	.48	.23

In addition to these examinations for base-exchange materials, the soils were examined to determine a number of other properties in respect to which the fertilizer and crop treatment might be expected to produce differences from the check plots. These determinations and the resulting values are given in Table 5.

TABLE 5.—*Miscellaneous chemical and physical determinations on Leonardtown silt loam*

Range No.	Plot No.	Organic matter	N	NO <sub>3</sub>	Organic matter Nitrogen	Carbon Nitrogen	P <sub>2</sub> O <sub>5</sub>	Soluble P <sub>2</sub> O <sub>5</sub> Total P <sub>2</sub> O <sub>5</sub>
1	1.....	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>			<i>Per cent</i>	<i>Per cent</i>
	2.....	2.08	0.087	0.0019	23.9	13.6	0.083	6.15
	3.....	1.75	.079	.0012	22.2	13.0	.058	2.08
	4.....	1.41	.060	.002	22.1	13.5	.050	1.00
2	1.....	1.91	.087	.0016	22.0	11.7	.114	4.21
	2.....	1.74	.078	.0041	22.3	13.0	.083	1.81
	3.....	1.69	.073	.0018	23.1	13.4	.074	.67
	4.....	1.89	.083	.0011	22.8	13.0	.145	3.51
3	1.....	1.75	.076	.006	23.0	13.3	.089	1.46
	2.....	1.21	.052	.0011	23.2	13.4	.067	.75
	3.....	1.91	.078	.005	24.5	13.0	.082	7.07
	4.....	1.80	.079	.005	22.7	13.2	.062	2.08
4	1.....	1.40	.061	.002	22.9	13.3	.054	1.47
Average of plots numbered 1.....		1.95	.084	.0024	23.3	12.8	.106	5.23
Average of plots numbered 2.....		1.76	.078	.0041	22.5	13.1	.073	1.86
Average of plots numbered 4.....		1.43	.061	.0017	22.8	13.4	.061	.97

<sup>5</sup> SCHOLLENBERGER, C. J., and DREIBELBIS, F. R. ANALYTICAL METHODS IN BASE EXCHANGE INVESTIGATIONS ON SOILS. Soil Sci. 30: 161-173, illus. 1930.

TABLE 5.—Miscellaneous chemical and physical determinations on Leonardtown silt loam—Continued

Range No.	Plot No.	Moisture equivalent	Colloid by mechanical analysis	Colloid by water-vapor absorption	Total base-exchange capacity per 100 g	Acidity per 100 g	Degree of saturation	pH
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Mg</i>	<i>Mg</i>	<i>Per cent</i>	
1	1.....	20.4	12.0	10.1	14.8	3.6	75.7	5.5
	2.....	20.0	11.9	9.6	14.3	3.36	76.5	5.8
	3.....	20.3	11.1	8.4	14.1	2.56	81.8	6.1
	4.....	20.3	10.6	8.8	15.0	2.92	80.3	5.8
2	1.....	20.4	11.7	8.7	14.4	1.82	87.2	6.9
	2.....	19.8	13.2	9.9	14.5	3.12	78.5	5.6
	3.....	19.9	12.5	10.9	15.6	3.04	80.5	6.1
	4.....	19.8	11.6	9.2	14.0	2.80	80.0	5.8
3	1.....	18.6	11.3	8.0	13.9	1.36	90.2	7.3
	2.....	21.2	11.2	8.3	14.1	2.72	80.7	5.9
	3.....	21.1	12.3	9.6	13.7	2.40	82.5	5.7
	4.....	19.5	10.8	7.8	13.2	2.48	81.1	5.6
	Average of plots numbered 1.....	20.4	11.6	9.5	14.9	3.07	79.3	-----
	Average of plots numbered 2.....	20.3	11.9	9.3	14.1	2.59	81.5	-----
	Average of plots numbered 4.....	19.5	11.6	8.5	13.9	2.38	82.9	-----

## DISCUSSION

The mechanical analyses of the samples (Table 2), although they show some differences, on the whole bear out the assumption of uniform texture throughout the area of the plots. Some divergence is to be expected owing to the conditions existing in the soil at the time of collection. Moreover, as Table 5 shows, the variations in organic matter affect the degree of dispersion, since not all the organic matter is removed by hydrogen peroxide.

The analyses of the acid extract (Table 3) reveal the fact that in general the extracted components of the check plots are slightly less than the corresponding materials from the fertilized plots. This was to be expected, particularly in the case of calcium and phosphorus because of the large additions of these materials. The addition of calcium did not, however, make the calcium content of the fertilized plots greater than that of the check plot in range 3. That so large a quantity of calcium and magnesium was actually present in this check plot is shown not only by its exceptional pH value as compared with the pH values of the other check plots, but also by a determination of the calcium carbonate content. This latter value is 0.07 per cent for the check plot in range 3 as compared with 0.02, 0.03, and 0.05 per cent for the other check plots. These quantities were determined by the method outlined by Alexander and Byers.<sup>9</sup> The presence of calcium carbonate may be owing to the result of an undue share of the added crushed limestone previously mentioned. Some adventitious change in this plot is evident in other respects also, though the differences have not been adequate to alter its general yields materially. This increased solubility of the components of the fertilized plots is relatively much greater in the case of the phosphorus pentoxide. There is, however, but little difference between the effects produced by rock phosphate and superphosphate. The

<sup>9</sup> ALEXANDER, L. T., and BYERS, H. G. A CRITICAL LABORATORY REVIEW OF METHODS OF DETERMINING ORGANIC MATTER AND CARBONATES IN SOILS. U. S. Dept. Agr. Tech. Bul. 317, 26 p., illus. 1932.

soluble phosphorus pentoxide of plots 1 bears about the same ratio to that of plots 2 as the phosphorus pentoxide of the rock phosphates added to plots 1 bears to the phosphorus pentoxide of the superphosphate added to plots 2. The rock phosphate added to plots 1 is equivalent to about 1,000 pounds of phosphorus pentoxide an acre, whereas the superphosphate added to plots 2 is equivalent to about 175 pounds an acre. There is, however, but little difference between the crop yields of plots 1 and plots 2. This would indicate that more rock phosphate was added to plots 1 than was necessary. It seems evident, therefore, if we compare Tables 1 and 3 that the outstanding characteristic of this area, so far as inorganic constituents are concerned, is a deficiency in phosphates.

The increase in solubility of the aluminum in the fertilized plots is marked and again is greater in the plot 1 series than in the plot 2 series, and is, in all cases, greater than the check plots in the same range of plots. The writer has no explanation to offer for the relatively much greater solubility shown by all the plots in range 2 (in corn when the samples were taken) and range 3 (in soybeans). The alterations in the solubility of silica are not marked by the action of the fertilizer additions, but the ratio of alumina to silica (8.91) is of an order that indicates free alumina or the decomposition of colloid by the acid. There seems to be no explanation in the history of the soil treatment for the greater solubility of silica and alumina in ranges 2 and 3.

The general trend for manganese and for potassium is in the direction of increased solubility with the more effective fertilizer treatment, but the alteration, as shown by the averages, is not very strongly marked and in both cases may be due to absorption of these elements from the added manure.

The data given in Table 4 for calcium, magnesium, and potassium show the same general relationships as those given in Table 3. The quantity of sodium dissolved is apparently somewhat greater but does not reach values of any special significance. On the other hand, the manganese dissolved by the ammonium acetate is approximately one-fifth to one-seventh of that dissolved by the 0.05 normal acid. The aluminum dissolved is but one-twentieth of that soluble in acid, and the iron and phosphoric acid content of the dissolved material are reduced to a trace. Schollenberger and Dreibelbis believe that "any aluminum found in ammonium acetate extract of soil may be presumed to have entered the solution by true exchange." If this is true, it follows that exchangeable alumina in the small quantities present is not toxic, since the plot 1 series have a somewhat higher content and are notably more productive than the check plots.

In Table 5 are shown a number of determinations that are significant. The addition of the manure has markedly increased the organic content, as measured by the combustion method, of the plots to which it was added; yet the increase is apparently less when the manure is associated with superphosphate. The organic matter in the check plots remains considerable, even though no addition has occurred, certainly not during the 10-year observation period and possibly for a much longer time. Yet the organic matter-nitrogen ratio shows no appreciable alteration due to increase of organic content, and the carbon-nitrogen ratio also is almost constant. It follows from these observations that the nitrogen content alters with the organic content.

In the nitrate content of the soils the only definite trend observed is in the check plots, where the nitrate content is much lower, except in range 2, from which the corn crop has just been harvested. There is no apparent explanation of this variation since the plots were all very dry and had remained so for many weeks before sampling. The determination of the total phosphoric acid accounts fairly accurately for the total phosphates added to both series of plots 1 and 2. The percentage of the total phosphate soluble in acid is strikingly greater in the plots treated with rock phosphate and makes very apparent the probable cause of the variation in yields shown in Table 1.

The moisture-equivalent measurements show no sharply altered water-holding capacity in the fertilized and unfertilized plots and are of about the order of magnitude that would be expected from the differences in organic-matter content. The quantity of colloid in the plots, as shown by mechanical analysis (0.002 mm fraction), indicates no alteration, and the quantity differences, as shown by the water-vapor absorption method, are such as might be expected from the known difference of absorption values of organic and inorganic colloids. Similarly, the base-exchange capacity of the plots is that which would be expected from the organic-matter differences in them.

In general the total exchangeable acids of the fertilized plots vary with the pH and average higher than in the check plots. The higher value in the check plot of range 2 is undoubtedly due to the addition of the manure to this range in the spring of the year in which the sampling was made. As a result of the increased quantity of organic matter in the fertilized plots, the mean values of the degree of base saturation is lower than in the check plots. These latter values also indicate that the degree of base saturation is sufficient to maintain good crop conditions, and they tend to confirm the comment previously made that the deficiency in this soil which limits production is the small content of available phosphorus.

The range of pH values shown by the soils of these plots is narrow and apparently gives no indication of productivity.

#### SUMMARY AND CONCLUSIONS

This paper reports a study carried out for the purpose of ascertaining the chemical changes in the soil colloid of a given soil type resulting from a treatment which changed the soil from a condition of comparative nonproductivity to one capable of increased crop yields. The data presented indicate that the only apparently essential alteration consists in the supply of available phosphorus. Whether the coincident increase in organic matter has had a part in rendering the phosphate available or whether the organic supply in the check plots is adequate, is not clear from the analytical evidence. It is also true that the fertilizer treatment given the plots has increased the average available potassium, manganese, magnesium, and calcium, and the total nitrogen content of the treated plots. It is not clear, however, that these alterations are of great significance; in particular it appears that increase in the available calcium with the consequent change in pH value of the check plot of range 3 is not followed by increased productivity.

The results of this study, which are in accord with those obtained by Holmes in a study of the colloid of Leonardtown silt loam, make it

clear that the plant food present, with the exception of phosphorus, is adequate for good crop yields.

It appears from the data obtained that, although N/20 hydrochloric acid and normal ammonium acetate are essentially equally effective in the removal of exchangeable bases from this soil, the effect of these reagents on the sesquioxide, phosphoric acid, and silica are very different. It does not seem possible, from the data, to draw any conclusions regarding the ratios of plant-food materials which favor productivity.

# OBSERVATIONS ON A SEVERE OUTBREAK OF MYCOSIS IN CHICKS<sup>1</sup>

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## INTRODUCTION

During the summer of 1931 the writer was called upon to investigate losses of chicks in a Connecticut hatchery that amounted to approximately 10,000 out of 50,000 hatched during the season, or about 20 per cent. This hatchery specialized in Single Comb White Leghorn pedigreed stock, and was in the process of eradicating pullorum disease according to the Connecticut official plan.

At first an unusual outbreak of bacillary white diarrhea was suspected, but detailed bacteriological examination of 45 chicks failed to reveal infection from this source. On further study the condition was found to be characterized by whitish lesions in the crop and by the presence of yeastlike fungi in the digestive tract. The disease was finally determined to be thrush, muguet (French), or Soorkrankheit (German).

## LITERATURE

According to Kaupp,<sup>2</sup> chickens and turkeys affected with thrush, aphtha, or sore mouth appear dull and emaciated and often die in convulsions. The mucous lining of the mouth and esophagus shows whitish or brownish-yellow adherent deposits consisting of microscopic hyphae and oval spores of *Oidium* (*Saccharomyces*) *albicans*. Van Heelsbergen<sup>3</sup> calls the disease oidiomycosis, and describes it as an affection of the mucous membrane of the mouth, fauces, esophagus, crop, stomach, and small intestine of birds, mammals, and man. Young chickens, pigeons, geese, and turkeys appear to be especially susceptible. However, according to Lahaye, as quoted by Van Heelsbergen,<sup>4</sup> thrushlike affections in pigeons are often caused by fowl-pox virus. In the description of stomatitis oidica of birds, Huttyra and Marek<sup>5</sup> quote Schlegel who observed cases in which the mouth cavity showed only discoloration, while the glandular stomach and gizzard revealed true thrush mycosis or ulcerlike foci.

On the whole, a study of the available literature leaves the impression that the disease is of epidemiological importance in pigeons but not in chickens.

The disease reported here appears to be different from the mycosis of birds described by Staub and Truche,<sup>6</sup> for in the writer's cases the

<sup>1</sup> Received for publication Apr. 11, 1932, issued February, 1933.

<sup>2</sup> KAUPP, B. F. POULTRY DISEASES AND THEIR TREATMENT. Ed. 5, rev. and enl., 436 p., illus. Chicago. 1929.

<sup>3</sup> HEELSBERGEN, T. VAN. HANDBUCH DER GEFLÜGELKRANKHEITEN UND DER GEFLÜGELZUCHT. 608 p., illus. Stuttgart. 1929.

<sup>4</sup> HEELSBERGEN, T. VAN. Op. cit.

<sup>5</sup> HUTTYRA, F., and MAREK, J. SPECIAL PATHOLOGY AND THERAPEUTICS OF THE DISEASES OF DOMESTIC ANIMALS. 3d authorized Amer. ed. from 6th rev. and enl. German ed., 3 v., illus. Chicago. 1926.

<sup>6</sup> STAUB, A., and TRUCHE, C. THE FIGHT AGAINST INFECTIOUS DISEASES OF POULTRY IN FRANCE. 4th World's Poultry Cong. Rpt. 1930 (Sec. C): 386-388. 1931.

heart, liver, and peritoneum did not show the typical whitish films. It likewise differed from mycosis of turkeys, said by some to be identical with oidiomycosis of chickens.<sup>7</sup> The writer<sup>8</sup> has found the former disease to be characterized by yellow, conical, slightly curved processes with a hemorrhagic areola. These young lesions may become confluent and form extensive ulcers in the crop, esophagus, and glandular stomach. Such pathological manifestations, aside from the unknown etiology of mycosis in turkeys, constitute, in the writer's opinion, a distinction between thrushlike affections of turkeys and chickens.

### EPIDEMIOLOGY

The plant in which the present observations were carried out was situated on a well-drained hilly plateau and spread out over considerable territory. Brooding was carried on in 9 by 12 foot colony houses which were kept rather warm during the summer. The disease first appeared in the second or third hatch of the season,

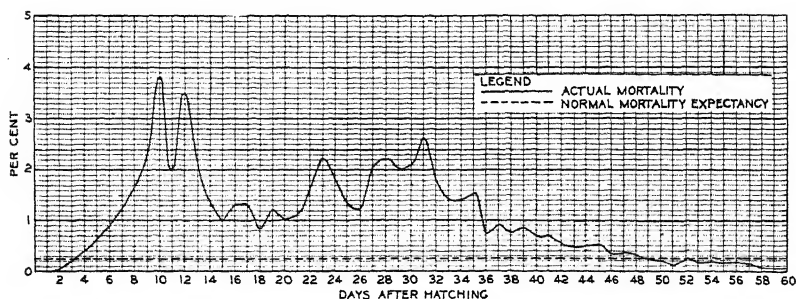


FIGURE 1.—Average daily mortality of 4,919 chicks during the first 60 days after hatching as compared with normal mortality expectancy

during the middle of April, increased in intensity during June and July, and disappeared at the end of August.

Clinical symptoms appeared from six to seven days after hatching and consisted of dullness, listlessness, huddling together, and marked roughness of the feathers. Gaping and diarrhea were not noticed as flock symptoms. Mortality started almost simultaneously with the appearance of clinical evidence, and reached a peak about the tenth day and again about the thirty-first day after hatching. Chicks that reached the age of 60 days had apparently recovered from the disease and developed into normal birds.

Daily records were kept of the mortality in 10 colony houses having a population of 4,919 chicks. From these records the total daily mortality was computed in terms of percentage and the data plotted as shown in Figure 1. For comparison, a corresponding line was plotted, based on a normally expected mortality of 15 per cent during the first 60 days after hatching. This latter figure was taken as the high average of a 3-year chick-mortality survey carried out in Connecticut.<sup>9</sup>

<sup>7</sup> HUTYRA, F., and MAREK, J. Op. cit. KAUPP, B. F. Op. cit. HEELSBERGEN, T. VAN. Op. cit.

<sup>8</sup> JUNGHER, E. TWO INTERESTING TURKEY DISEASES. Jour. Amer. Vet. Med. Assoc. 71: 636-641, illus. 1927.

<sup>9</sup> JONES, R. E. THREE YEARS' GROW HEALTHY CHICKS SURVEY FIGURES. Conn. Agr. Ext. Serv., Home Egg Laying Contest v. 10, 1st issue November. 1928.



The mortality in the 10 colony houses under observation varied considerably, as is shown in Figure 2. The thin lines represent individual colony houses, the numbers indicating the population. The heavily stocked colony houses appear to have suffered most, and overcrowding may therefore be considered a factor in the spread of the

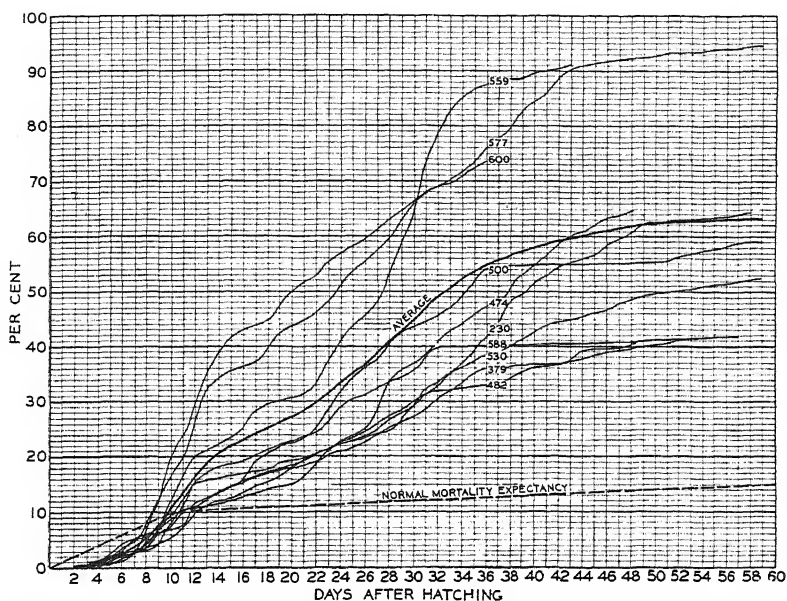


FIGURE 2.—Mortality of 4,919 chicks in 10 colony houses during the first 60 days after hatching, as compared with normal mortality expectancy; numbers indicate population of colony houses; heavy line shows total average mortality

disease. The thick line represents a total average mortality of 63 per cent.

During the ravages of the disease various changes in management were made in an attempt to check the losses. The results are shown in Table 1.

TABLE 1.—Comparison of loss of chicks reared under different conditions

Point of comparison	Condition of rearing	Number of chicks	Number died	Per cent of loss during first 60 days of life
Eggs and incubators...	Native <sup>a</sup> eggs hatched in native incubators.....	3,858	2,606	67.5
	Native eggs hatched in foreign <sup>a</sup> incubators.....	760	424	55.8
	Foreign eggs hatched in foreign incubators.....	302	99	32.8
Sanitation in colony house.....	Chicks in colony houses cleaned once a week.....	559	514	91.8
	Chicks in colony house cleaned once a day.....	588	243	41.3
	Chicks in colony house on wire floors.....	482	202	41.9

<sup>a</sup> Native=on the original hatchery plant; foreign=away from the original hatchery plant.

It will be seen that a change in incubators brought about a slight decrease in mortality, but certainly not enough for all the losses to be attributed to incubation. Even chicks from an entirely different

source became affected by the disease when brought to the premises, suffered a loss of 32.8 per cent. Neither a daily cleaning of the colony houses nor the use of wire floors was very effective in controlling the disease, for the losses under strictly sanitary conditions amounted to 41.9 per cent.

The total actual mortality in this outbreak can only be estimated, since about 90 per cent of the total output of 50,000 was sold as day-old chicks. According to the original owner, the purchasers reported losses aggregating 6,000, under circumstances very closely resembling those observed on the home plant. While there is no direct evidence that all of these 6,000 chicks died from the same cause, the writer found the characteristic lesions of thrush in the organs of five abnormal chicks selected at random from a group that had been purchased from the home plant. The losses were reported principally by buyers of large lots; the buyers of small lots, or rather those who brooded the chicks in small flocks or batteries, apparently had better luck. The widespread reports of losses would indicate that feeding methods, which were known to be quite varied among the owners, could not be considered an important factor in the trouble.

#### PATHOLOGY

In a study of the disease it was surprising to find that gross lesions in chicks from 2 to 3 weeks old were often so small as not to be easily distinguishable. In older chicks gross lesions tend to become localized in the crop and are readily recognized. In 120 field cases recorded, 84 showed lesions in the crop, 29 in the proventriculus, 53 in the gizzard, 6 in the liver, and 44 in the gall bladder; in 12 field cases no macroscopic lesions were observed.

In very young chicks the gross lesions in the crop consisted of pinhead-sized whitish nodules, and in older chicks of circular whitish ulcerlike patches (fig. 3, A) ranging in diameter from 1 to 5 mm., or of grayish-white pseudomembranes often forming a ridge along the folds (fig. 3, B). Very often only loose necrotic material with no apparent injury to the underlying tissue was found. As a rule, the unopened crop appeared tympanitic on palpation.

A microscopic examination of the crop showed extensive destruction of the stratified epithelium deep in the Malpighian layer (fig. 3, C), and quite often walled-off ulcers (fig. 3, D) or extensive diphtheroid to diphtheritic membranes. The lesions were characterized by the absence of inflammatory reaction.

The proventricular wall appeared at times diffusely swollen, or exhibited whitish, opaque, circular areas on the serous surface. The mucous surface was covered with brownish or mucoid exudate. Under the microscope necrotic lesions were seen in the epithelium of the superficial glands and in the centers of small lymph nodes. (Fig. 4, A and B.)

The corneous lining of the gizzard frequently revealed ulcers (fig. 4, C), especially in the cardiac region (fig. 4, D), or old hemorrhages which were visible by transmitted light (fig. 4, E). The true mucous membrane of the gizzard had usually lost its normal glossiness and appeared opaque and grayish white, or it showed whitish shallow ulcers. On microscopic examination, the ulcers in the lining were found to consist mostly of craters in the tissue, with little cellular reaction around them.

In the majority of cases the liver was normal, although occasionally whitish pinhead-sized nodules or small hemorrhagic infarcts were seen. Sections of some livers which appeared normal to the naked eye showed, under the microscope, periportal focal necrosis. (Fig. 5,

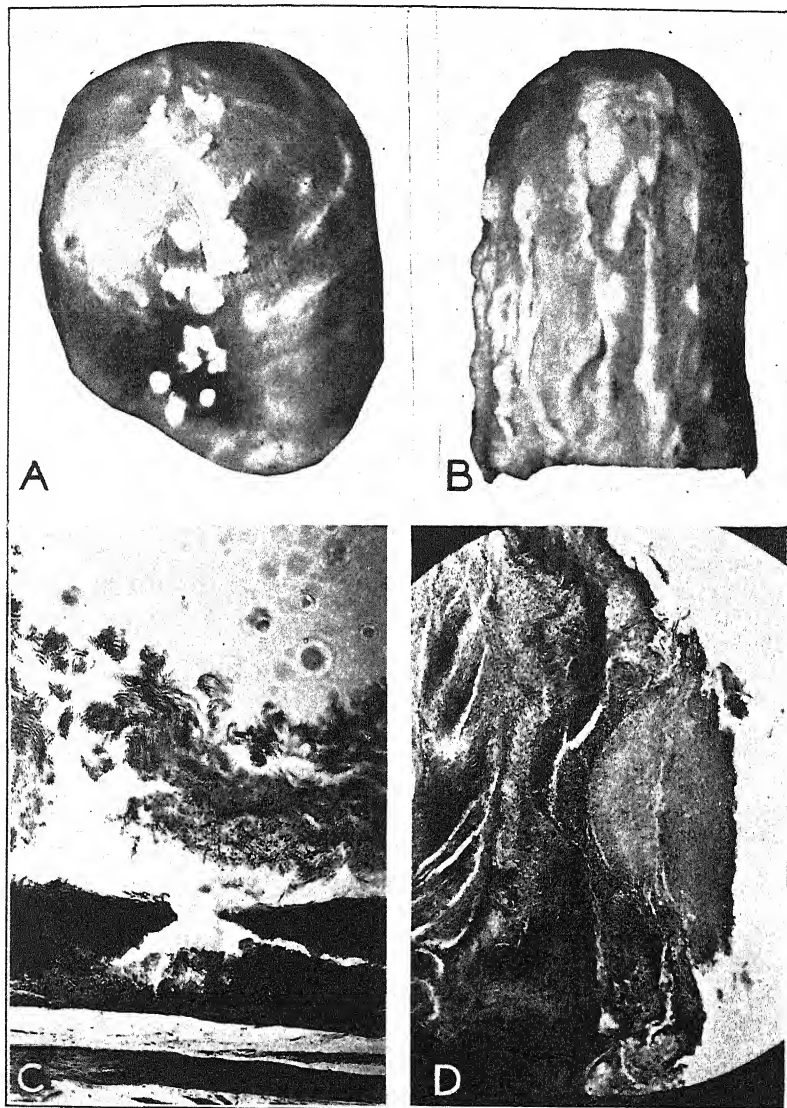


FIGURE 3.—A, Inverted crop No. 1513, showing circular mycotic lesions,  $\times 3$ ; B, inverted crop No. 1488, showing mycotic lesions along the folds,  $\times 3$ ; C, section of crop No. 1500, showing extensive destruction of stratified epithelium,  $\times 100$ ; D, section of crop No. 1642, showing walled-off ulcer,  $\times 70$

A.) The gall bladder was often enlarged and the bile thickened. The duodenum appeared congested and hemorrhagic, and the caecal contents foamy. In some instances the caeca contained large numbers of a protozoon which morphologically resembled *Trichomonas*

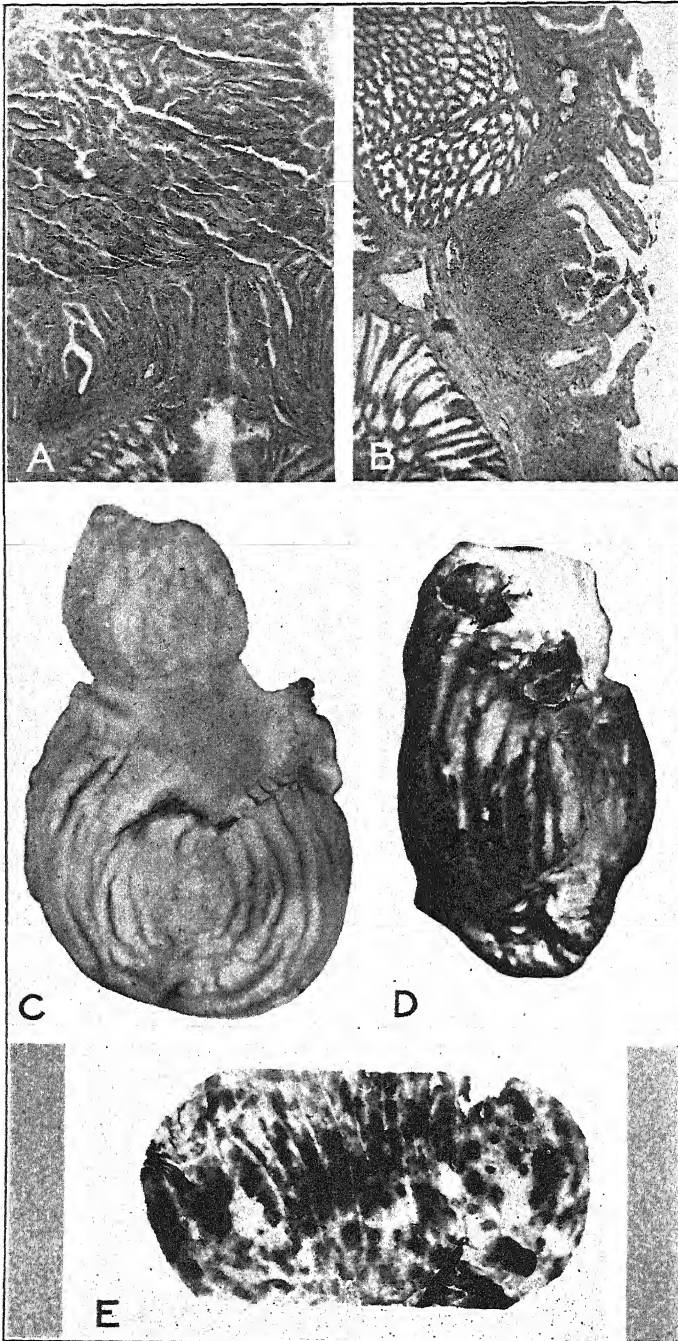


FIGURE 4.—A, Section of proventriculus No. 1732, showing necrosis of epithelium of superficial glands,  $\times 70$ ; B, section of proventriculus No. 1731, showing focal necrosis of lymph nodule,  $\times 70$ ; C, inverted gizzard No. 1596, showing superficial ulcer in corneous lining,  $\times 2$ ; D, inverted gizzard No. 1673, showing circular ulcers at cardia,  $\times 2$ ; E, corneous lining of gizzard No. 1772, photographed by transmitted light, and showing old hemorrhages and whitish, shallow ulcers,  $\times 1.5$

*eberthi*; occasionally they contained hemorrhagic cores which were probably due to an intercurrent infection with *Eimeria tennella*. The kidneys often appeared pale and slightly tumefied. The ureters were distended.

#### ETIOLOGY

In the literature<sup>10</sup> the statement is frequently made that hyphae can be demonstrated readily in scrapings of lesions. In the present study mycelial threads were found infrequently, but spores could be seen, although recognition of them as such was attended with some difficulty.

Since cultural studies on routine laboratory media were inconclusive, the use of Sabouraud agar was resorted to. From 64 field cases that were cultured yeastlike fungi were isolated in 54 instances from various organs, such as the crop, proventriculus, gizzard lining, true mucous membrane of the gizzard, the liver, gall bladder, and intestine. As a rule, only one type of organism was isolated from any one organ.

The types of yeastlike fungi isolated fall into three main groups, according to their growth characteristics on Sabouraud agar. The first type, which was isolated 38 times, produced a dry, whitish, high convex colony, consisting of spherical conidia with little or no mycelial structures in young cultures. This organism produced general infection in rabbits, on intravenous injection, and according to Benham<sup>11</sup> resembled *Monilia albicans*. The second type, which was isolated 16 times, developed a rough, feathery, adherent colony, consisting of thick mycelium and spores. It was not pathogenic to rabbits and resembled *Oidium lactis*. The third type, isolated fifteen times, produced a smooth, grayish, flat colony, consisting of small elliptical conidia. It was not pathogenic to rabbits and resembled *Monilia krusei*.

In an effort to reproduce the disease by artificial infection, tests were made with 52 chicks ranging in age from 3 to 22 days. Five chicks were fed with a composite fecal specimen from field cases. Four of the chicks succumbed to the infection. (Table 2.) All showed typical lesions in the crop (fig. 5, B) and one of them in the mouth, also. This was the only mouth lesion seen during the outbreak. *Monilia albicans* was isolated from the principal lesions. This experiment proved rather conclusively that droppings from diseased birds are infectious and that *M. albicans* must be considered the principal cause of the disorder. To test the pathogenicity of pure cultures, 47 of the 52 chicks were injected in different ways. *M. albicans* was found to be highly pathogenic, *Oidium lactis* much less so, and *M. krusei* was apparently not harmful under experimental conditions. (Table 2.)

The method of administration, whether oral, subcutaneous, intravenous, or intraperitoneal, did not seem to be of particular importance in the demonstration of pathogenicity, but the age of the chick appeared to be a definite factor, chicks from 1 to 2 weeks old being most susceptible.

<sup>10</sup> HUTYRA, F., and MAREK, J. Op. cit. KAUPP, B. F. Op. cit. HEELSBERGEN, T. VAN. Op. cit.

<sup>11</sup> BENHAM, R. W. Private communication. 1931.

In these experiments the time from artificial infection to death ranged from 10 to 45 days, the average being 31 days. It should be kept in mind, however, that these experiments took into account

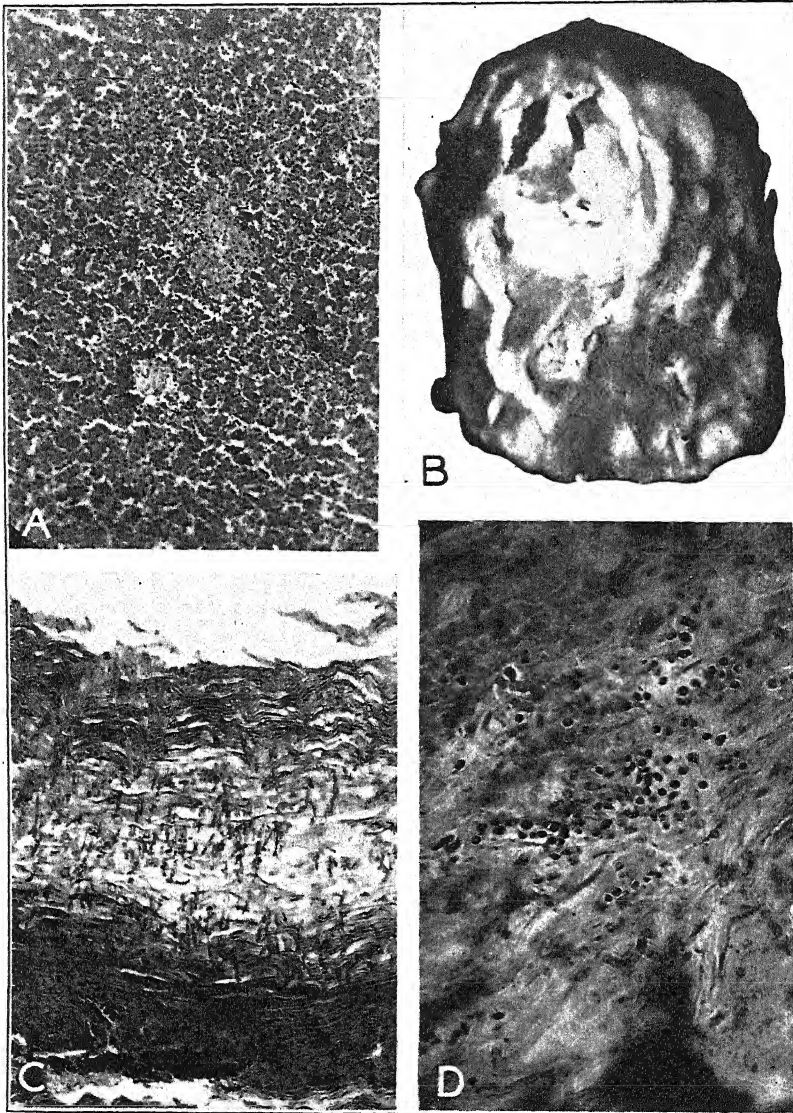


FIGURE 5.—A, Section of liver No. 1724, showing focal necrotic areas,  $\times 140$ ; B, inverted crop No. 1586, showing lesions produced in chick by feeding fecal material from infected birds,  $\times 3$ ; C, section of crop No. 1621, showing numerous mycelial structures in stratified epithelium,  $\times 200$ ; D, section of crop No. 1488, showing clusters of spores in stratified epithelium,  $\times 600$

only actual mortality and not morbidity, which is known from field observations to be always considerably higher.

In a further study of the mode of transmission of the disease 100 eggs were hatched in sterilized incubators and the chicks reared in



sterilized battery brooders. In the crops of four of these chicks characteristic lesions of thrush were found and *Monilia albicans* isolated from them. Some of the hens from the poultry plant suffered from an extremely moist type of vent gleet, and a mycological examination of lesions from these birds yielded fungi of the *M. albicans* type.

TABLE 2.—Results of experimental feedings of or injections with infectious material

Infectious material	Number of chicks infected	Fatal cases
Feces.....	5	4
Pure cultures resembling—		
<i>Monilia albicans</i> .....	16	11
<i>Oidium lactis</i> .....	16	2
<i>Monilia krusei</i> .....	15	0

As a final link in the evidence of the etiological relationship of yeastlike fungi to the disease outbreak, mycelial structures (figs. 3, C, and 5, C), or clusters of spores (fig. 5, D), were often demonstrated in microscopic sections of the organs.

#### DISCUSSION AND SUMMARY

Losses totaling 10,000 chicks were observed in a commercial hatchery of 50,000 chicks that were less than 60 days old. The average loss in the home plant was 63 per cent, and in the chicks that were sold as day old, 13 per cent. The morbidity was much higher, according to the clinical appearance of the flocks. The chicks that survived made good recovery, and 60 days after hatching had developed into normal birds. Losses were smaller when brooding was carried out in small lots or batteries; change in feed and rigid sanitary precautions were not very effective as control measures.

The diseased condition was characterized by whitish ulcers or pseudomembranes in the crop, brownish or mucoid deposits in the proventriculus, and ulcers in the gizzard. The lesions in small chicks were often so small as to be easily overlooked.

On mycological examination yeastlike fungi were isolated from the crop, proventriculus, gizzard, gall bladder, and intestine. The predominating organism was a yeastlike fungus resembling *Monilia albicans*; the other observed types resembled *Oidium lactis* and *M. krusei*.

The diseased condition was reproduced by feeding fecal material from diseased chicks and by injecting fungus cultures of the *Monilia albicans* type. In two instances artificially infected chicks succumbed with the *Oidium lactis* type. Infection experiments conducted on small lots of chicks were not uniformly successful, a point readily understood when field observations of small-lot brooding are considered. The average period of incubation under experimental conditions was 31 days, a period that closely coincided with the second mortality peak under field conditions. The second mortality peak may, therefore, have been due to postnatal infection.

The disease may appear in septicæmic form, as was indicated by the isolation of virulent fungi from the liver and gall bladder; how-

ever, the extreme virulence of the infection in very young chicks, together with the slight pathological changes that occurred and the occasional presence of focal necrosis in the liver, suggests a toxemic action of the fungi.

Day-old chicks from the hatchery developed the disease after they had been removed from the original premises. Chicks raised on wire floors in a new, isolated colony house suffered 41.9 per cent losses. The first and highest mortality peak occurred about the tenth day after hatching; the average incubation period under experimental conditions was 31 days. The cloaca of laying hens affected with a moist type of vent gleet was shown to carry yeastlike fungi. When eggs from the plant were hatched in a sterilized incubator and the chicks reared in sterilized battery brooders, the disease appeared in only a few instances. These data furnish circumstantial evidence that the disease can be transmitted through the agency of the egg, presumably on the egg.

The fact that the owner found that brooding in batteries was safer than in colony houses, and the writer's observation that only relatively small numbers of chicks succumbed to experimental infection, suggest that management may play a part in the postnatal spread of the disease.

According to available information, this report of a severe outbreak of thrush or mycosis in chicks is the first in which the disease has been shown to be of major epidemiological importance.



# THE EFFECT OF DIFFERENT SOAPS ON LEAD ARSENATE IN SPRAY MIXTURES <sup>1</sup>

By JOSEPH M. GINSBURG

*Biochemist in Entomological Investigations, New Jersey Agricultural Experiment Station*

## INTRODUCTION

Among the various chemicals employed to increase the spreading properties of insecticides on foliage, soaps undoubtedly rank as the most efficient and cheapest. Yet soaps can not be safely used with sprays containing arsenicals because the chemical reaction involved in such mixtures tends to liberate soluble arsenic, often in quantities large enough to produce injury to the foliage. It has been shown by Tartar and Bundy <sup>2</sup> and Pinckney <sup>3</sup> that acid lead arsenate ( $\text{PbHAsO}_4$ ) produces much more soluble arsenic than the basic lead arsenate ( $\text{Pb}_5\text{OH}(\text{AsO}_4)_3$ ) when mixed with soaps. Pinckney has also shown that a stearate soap forms considerably more soluble arsenic than does an oleate soap when mixed with either the basic or the acid lead arsenate.

The differences observed between the two fatty acids mentioned above suggested that soaps consisting of the same fatty acid but of different bases might exhibit similar or even greater differences in their action on lead arsenate. Thus, soaps having a strong base, such as sodium or potassium, might produce more soluble arsenic and consequently greater injury to foliage than soaps having a weak base, such as ammonium, triethanolamine, etc. If such should be the case it might be possible to prepare a soap from oleic acid and a very weak base which would be compatible with lead arsenate spray mixtures and safe to use on foliage. Accordingly, soaps of different bases were tested in field and laboratory experiments.

## FIELD TESTS

During July, 1930, several apple trees were sprayed with mixtures consisting of acid lead arsenate, hydrated ferric oxide, and soap. The proportions of the ingredient per 100 gallons of spray were as follows: 3 pounds lead arsenate, 4 pounds ferric oxide, and 0.5 per cent soap. In previous tests it had been found that this amount of soap was sufficient to produce good spreading of the lead arsenate on apple foliage. The ferric oxide was added as a sticker and corrective. The ability of this chemical to increase the adhesion of lead arsenate and prevent arsenical injury to foliage has already been shown. <sup>4</sup> The three soaps selected for the test were commercial potash fish-oil soap, potassium oleate, and triethanolamine oleate. The last two soaps

<sup>1</sup> Received for publication Apr. 6, 1932; issued February, 1933. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

<sup>2</sup> TARTAR, H. V., and BUNDY, L. A. SOLUBLE ARSENIC IN MIXTURES OF LEAD ARSENATE AND SOAP, *Jour. Indus. and Engin. Chem.* 5: 561-562. 1913.

<sup>3</sup> PINCKNEY, R. M. ACTION OF SOAP UPON LEAD ARSENATES. *Jour. Agr. Research* 24: 87-95. 1923.

<sup>4</sup> GINSBURG, J. M., and MANN, R. F. STUDIES WITH HYDRATED FERRIC OXIDE AS CORRECTIVE AND STICKER FOR LEAD ARSENATE AND NICOTINE TANNATE. *Jour. Econ. Ent.* 24: 695-701. 1931.

were prepared in the laboratory from pure chemicals. The first two soaps contain a strong, highly dissociating inorganic base (K), while the third contains the comparatively weak organic base  $(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}$ . The ingredients were mixed in the following manner: The lead arsenate and ferric oxide were first made into a thin paste by the addition of a small amount of water. The rest of the water containing the required amount of soap was then added. The spray was applied from a 3-gallon bucket pump. The trees received three sprays at 2-week intervals.

TABLE 1.—Results of spraying apple trees with lead arsenate and ferric oxide, with different soaps as spreaders, 1930

Soap used	Injury observed on trees sprayed—			Nature of injury
	July 3	July 17	July 30	
Potassium oleate.....	Very slight....	Slight.....	Appreciable *	Brown leaf areas.
Triethanolamine oleate.....	None.....	None.....	Slight *.....	A few brown leaf tips.
Potash fish-oil soap (commercial).	Appreciable....	Severe.....	Severe *	About 30 per cent defoliation
Check (no soap).....	None.....	None.....	None *.....	

\* Observations made on Aug. 10.

The results, which are presented in Table 1, show that arsenical injury from the sprays containing either fish-oil soap or potassium oleate was apparent after the first application. The injury from fish-oil soap was more pronounced and increased severely with each subsequent spraying, until by August 10 there was about 30 per cent leaf drop. The potassium oleate produced brown spots on the leaves but did not cause defoliation. The trees sprayed with triethanolamine oleate showed no injury until after the third application and even then injury was slight as compared with that produced by the potassium soaps. There was no visible arsenical injury on the check trees sprayed with lead arsenate and ferric oxide only.

#### LABORATORY TESTS

Soaps were prepared from pure oleic acid with the following bases: Sodium, potassium, ammonium, and triethanolamine  $(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}$ . In addition to these pure soaps, a commercial fish-oil soap was used. Samples of acid lead arsenate in concentrations equivalent to 3 pounds in 100 gallons of spray were mixed in flasks containing 500 c c of distilled water with various dilutions of soaps (0.25, 0.5, 1 per cent) and allowed to stand for 24 hours, with frequent shaking. At the end of this period the soluble arsenic was determined by the procedure outlined in the official methods of the Association of Official Agricultural Chemists.<sup>5</sup> The only deviation from this method was that the mixtures were kept at room temperature instead of at 32° C. The lead arsenate was a commercial brand, which contained 32.4 per cent  $\text{As}_2\text{O}_5$ .

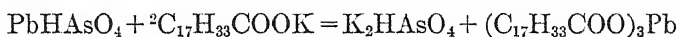
<sup>5</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

TABLE 2.—*Soluble arsenic liberated from 1.8 g lead arsenate mixed in 500 c c of water with various concentrations of soaps*

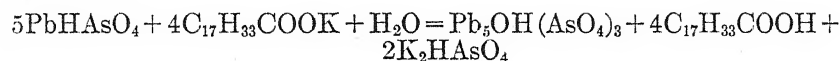
Soap used	Concen- tration of soap	Soluble As <sub>2</sub> O <sub>3</sub> formed	Lead arsenate in solu- tion
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Potash fish-oil soap (commercial) -----	0.25	13.32	47.28
	.50	20.87	74.10
	1.00	21.56	76.50
	.25	9.25	32.83
Sodium oleate -----	.50	10.80	38.34
	1.00	16.79	59.60
	.25	8.15	28.93
Potassium oleate -----	.50	9.78	34.71
	1.00	10.94	38.83
	.25	5.39	19.13
Ammonium oleate -----	.50	6.67	22.78
	1.00	6.14	21.80
	.25	3.83	13.59
Triethanolamine oleate -----	.50	4.63	16.44
	1.00	5.59	19.84
Check (lead arsenate only) -----		.43	1.53

The results, presented in Table 2, clearly show that soaps of strong bases liberate more soluble arsenic than soaps of weak bases. Thus, ammonium oleate gave considerably less soluble arsenic than the oleate of either potassium or sodium, while the triethanolamine oleate, the weakest base tested in this experiment, gave the lowest amount of soluble arsenic.

A comparison of the results in Table 2 brings out the fact that the amount of lead arsenate decomposed is not in direct proportion to the amount of soap present in solution. Thus, 0.25 per cent potassium oleate decomposed 28.93 per cent lead arsenate, while 1 per cent of the same soap decomposed only 38.83 per cent. Again, 0.25 per cent ammonium oleate dissolved 19.13 per cent, of the lead arsenate, while 1 per cent ammonium oleate dissolved only 21.80 per cent. Evidently the reaction does not go to completion and can not be represented by the simple formula



The reaction is more probably of double decomposition, as suggested by Smith.<sup>6</sup>



The results from the laboratory tests fully explain why considerably more injury to apple foliage resulted from arsenical mixtures containing potassium oleate than from those containing triethanolamine soap. The former produced more than twice as much soluble arsenic as did the latter. These results further explain why most of the foliage injury was produced by the spray containing fish-oil soap. This soap decomposed more lead arsenate than any one of the other soaps. Evidently the amount of hydrated ferric oxide used in the field tests was not sufficient to take care of the large quantities of soluble arsenic liberated from the lead arsenate by the fish-oil soap. It is clear from

<sup>6</sup> SMITH, G. E. LEAD ARSENATES. A STUDY OF THE FACTORS CONTROLLING THE REACTIONS OF LEAD NITRATE AND LEAD ACETATE WITH DISODIUM ARSENATE. Jour. Amer. Chem. Soc. 38: 2014-2027. 1916.

the results here shown that soaps, if used at all with lead arsenate, should not consist of a mixture of fatty acids, such as those present in fish oil, but should be prepared from pure oleic acid and a weak base.

#### SUMMARY OF RESULTS

Apple trees were sprayed three times at 2-week intervals with mixtures consisting of lead arsenate, hydrated ferric oxide, and each of the following soaps: Commercial potash fish-oil soap, potassium oleate, and triethanolamine oleate.

The spray containing fish-oil soap produced severe injury and caused about 30 per cent leaf drop. The injury from the potassium oleate spray was limited to spots on the leaves and browning of the leaf edges. The injury from the triethanolamine oleate spray was very slight and did not appear until after the third application.

Simultaneously with the field tests the action of the following soaps on lead arsenate was tested in the laboratory: Potash fish-oil soap, sodium oleate, potassium oleate, ammonium oleate, and triethanolamine oleate. The results indicate that soaps of strong bases form more soluble arsenic than soaps of weak bases. Of all the soaps tested, potash fish-oil soap produced the greatest quantity of soluble arsenic and triethanolamine oleate the least.

# A METHOD FOR DETERMINING THE SPECIFICITY OF THE INTRACELLULAR GLOBULIN OF *FUSARIUM LINI*<sup>1</sup>

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## INTRODUCTION

Groups and species of plants and animals have been successfully differentiated by serological means through the use of protein fractions from these organisms. The success of such methods of differentiation depends in part upon the natural specificity of the protein obtained and the sensitiveness of the technic used in demonstrating this specificity. Such technic, while it has not received universal sanction, has proved successful in some practical ways. Success with this type of work will be more common when the methods devised are less destructive of specific factors which are characteristic of the protein fractions. Globulins and other specific protein fractions are extremely sensitive to alteration, whether it be imposed by laboratory practice or by physiological factors, such as disease and disturbed metabolism. Indications of these alterations are numerous in syphilology and animal pathology. There are indications that variations in globulin may accompany disease even in plants (5).<sup>2</sup>

By means of direct and cross titrations of antisera produced with globulin antigens from various varieties of flaxseed it has been possible to differentiate the flax strains on the basis of wilt resistance (8). By analogous means it has been possible to classify various wheat hybrids relative to their interrelationships (7). In both cases protein fractions corresponding to the so-called globulins obtainable from antigenic materials were used to produce the antisera for the titrations.

## THE PROBLEM

Varieties of flax resistant to the wilt organism (*Fusarium lini* Bolley) in one locality have not proved equally resistant in other localities. Frequently these localities are not sufficiently dissimilar for the differences in resistance to be explained on the basis of climate alone. Broadfoot (1) has suggested that they may be due to physiological variations in the parasitic fungus. If this is the case, physiologic strains must be characterized by more than morphologic factors—presumably by biochemical ones.

The author has never found described in the literature a satisfactory method for studying the protein content of fungus material. It is generally stated that mycelium is meagerly supplied with protein. Heck (6) suggests a ratio of carbon to nitrogen of about 7 or 10 to 1. This ratio is largely determined by the composition of the culture medium. The usual methods fail to demonstrate the full protein content of the mycelium. Before physiologic strains of fungi can be differentiated through the use of intracellular proteins, a method for

<sup>1</sup> Received for publication Apr. 11, 1932; issued February, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 187.

obtaining these proteins must be developed, and their specific antigenic nature demonstrated. A method suitable for such a purpose is presented in this report.

### CULTURES

The cultures used were strains of *Fusarium lini* which had been isolated at the University of Minnesota. These were grown on hard potato agar and on corn-meal agar. All transfers were made in an inoculation room; stock transfers were made with a bit of mycelium, but for general purposes a cubic centimeter of young culture was drawn from a flask with a sterile pipette and used to inoculate liquid media. Erlenmeyer flasks of 250 c c capacity were used for culturing the fungus. The incubation temperature was about 28° C.

It was difficult to obtain a satisfactory type of growth in a reasonable length of time, that is, in three to four weeks. In trying to do this, various synthetic media were used, such as Czapek's sucrose nitrate solution, Richards' solution, peptone broth, and Raulin's solution. Of these, Raulin's solution gave the most satisfactory growth.

The most important consideration in this work was the amount of carbohydrate available in the medium. If too little was present the growth was feeble and yielded little protein, if too much the fungus rapidly reached maturity and converted the excess carbohydrate into reserve stores of fat. Such synthesis of fats was followed by a considerable lag in the growth.

Growth on Raulin's solution consisted of mats of mycelium and spores. Three types of spores were observed, microspores, macrospores, and chlamydospores. Of these the most numerous were the microspores. Young growth 2 to 3 weeks old was used, since it was found that such material was more easily extracted and gave a better yield of protein than similar amounts of older material. Young material was relatively free from storage fat, which could always be detected in older cultures, even without an excess of carbohydrate.

### EXTRACTION OF PROTEIN

Two methods of extraction were developed. (1) The mycelial mats were drained and then ground in a ball mill for from 36 to 48 hours in a 5 per cent solution of sodium chloride. The macerated mass was centrifuged at 3,000 revolutions per minute for 10 to 20 minutes, and the opaque liquid was then electrodialyzed in a Bronfenbrenner apparatus. The resulting precipitate gave a very weak biuret reaction. The mycelial residue, alkalized with N/100 NaOH gave a strong biuret reaction, indicating that the method had not satisfactorily extracted the protein fraction from the mycelial mass.

(2) A method suggested by the work of Chibnall (2, 3, 4) was next used. Mats of mycelium 3 to 4 weeks old were drained and dried for 3 to 4 hours at 35° C. in an incubator room. They were then immersed in ether for 1½ to 2 minutes. Ether was removed by evaporation in the incubator room for 5 to 6 hours. The mycelial mass was then placed in muslin sacks and subjected to heavy pressure in a screw press. A clear brownish liquid was expressed which gave a strong positive biuret reaction. According to Chibnall, this liquid is called "cell sap." It was electrodialyzed at between 20° and 30°, the process taking from 1½ to 4 hours. Before electrodialysis, the pH

value of this cell sap was 5.6; after dialysis it was 7.4 to 8. The precipitated material in the electrodyalyzer, presumably now an iso-electric globulin, was held in very fine suspension, necessitating concentration in a centrifuge. The solid materials thus obtained were dissolved in 1 per cent sodium chloride. This solution was used for sensitization of rabbits and also as an antigen for precipitin titrations. The mycelial residue left after pressing was ground in the ball mill for 36 hours with 1 per cent sodium chloride. This material without further purification or electrodyalysis was also used for sensitizing rabbits. All materials were stored in a refrigerator during the progress of the experiment.

#### SENSITIZATION OF ANIMALS

The half-grown rabbits used had never been fed flax. They showed very little variation in weight during the period of sensitization. As stated above, two types of antigen solution were used, namely, purified globulin, in 1 per cent saline, and mycelial residue, in 1 per cent saline. The purified globulin in 0.5 c c to 1.0 c c amounts was injected at 1 to 5 day intervals, the smaller amount being given at shorter intervals than the larger amounts. The mycelial residue was injected in 0.25 c c to 1 c c amounts. All injections were intraperitoneal. The total period of injections was about 20 days. No local infection resulted from either type of injection. A strong Arthus's phenomenon was observed in connection with mycelium when this matter escaped subcutaneously. Absorption of such material was evidently very slow.

#### SERUM TITRATIONS

Five days after the last injection 15 c c of blood was obtained from the sensitized rabbits and checks by slitting a vein in the ear with a small sterile blade. The blood was kept in the refrigerator overnight and the serum was then separated from the clot and dilutions made in a 0.9 per cent sodium chloride solution as indicated in titrations. The concentration of antigen was held uniform.

In the first experiment the quantity of available antigenic material (purified globulin) was so small that all titrations were made in test tubes of 0.25 c c capacity. The total volume used in each tube was 0.04 c c made up of 0.02 c c of antigen and 0.02 c c of serum in different dilutions. Precipitates were observed by using a lens of 10 × magnification. Characteristic mealy gray precipitates were obtained.

As shown in Tables 1 and 2, spores of *Fusarium lini* did not agglutinate when either sera in several dilutions was added to a suspension of the spores and observed microscopically. The quantitative estimation of agglutination was not possible since the spores precipitated by gravity.

TABLE 1.—*Antigens versus homologous antisera, using microtechnic*<sup>1</sup>

Antigen	Precipitate at serum dilutions of—			
	1:20	1:40	1:80	1:160
Mycelium.....	+	++	+++	++++
Globulin.....	++	+++	++++	++++

<sup>1</sup> Plus signs have been used to indicate the quantity of precipitate as a measure of degree of specificity.

TABLE 2.—*Mycelium antiserum and globulin antiserum versus mycelium antigen, using microtechnic*<sup>1</sup>

Antiserum	Precipitate at serum dilution of—						
	1:20	1:40	1:80	1:160	1:320	1:400	1:640
Mycelium.....	+++	++	++	++	+	0	0
Globulin.....	+++	++	++	++	0	0	0

<sup>1</sup> Plus signs have been used to indicate the quantity of precipitate as a measure of degree of specificity.

When a larger supply of antigen became available titrations were repeated. In these titrations the total volume of liquid in each test tube was 1 c c, made up of 0.5 c c of antigen and 0.5 c c of serum dilution. The results (Table 3) agree with those obtained in the first experiment in which minute amounts of reagent materials were used.

TABLE 3.—*Mycelium antiserum and globulin antiserum versus mycelium antigen, using larger volumes*<sup>1</sup>

Antiserum	Precipitate at serum dilution of—						
	1:20	1:40	1:80	1:160	1:320	1:400	1:640
Mycelium.....		+	+	+	0		
Globulin.....		++	++	++	+		
Normal serum ...	0	0	0	0	0	0	0

<sup>1</sup> Plus signs have been used to indicate the quantity of precipitate as a measure of degree, of specificity.

A comparison of Table 3 with Tables 1 and 2 shows no superiority in titration results where larger amounts of material were used.

The tables indicate that: (1) Both globulin and the mycelial residue from which the globulin was obtained possess specific antigenic properties. (2) Globulin is a more reactive antigen than the mycelial residue in homologous titrations. (Table 1.) (3) Globulin is a more reactive antigen than mycelial residue even when its antiserum is titrated against the heterologous antigen mycelial residue. (Table 2.) (4) When titrations were repeated with larger quantities of material the globulin antigen still possessed greater specificity. (Table 3.)

### CONCLUSIONS

A method is described by which specific antigenic protein material may be obtained from mycelial masses of *Fusarium lini*. A fraction corresponding to globulin possesses antigenic specificity in a higher degree than prepared mycelial masses. It is suggested that in proportion as the method fails to extract globulin from the mycelial mass the mycelial mass retains antigenic specificity. Further refinement of the technic of obtaining globulin from a mycelial mass of *F. lini* is necessary before it will be possible to check the interrelationships of the physiologic strains by serological methods.



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## PSYLLID YELLOWS OF THE POTATO<sup>1</sup>

By B. L. RICHARDS, *Botanist and Plant Pathologist, Utah Agricultural Experiment Station*; and H. L. BLOOD, *Plant Pathologist, Utah Agricultural Experiment Station, and Agent, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*<sup>2</sup>

### INTRODUCTION

In August, 1927, Richards, Blood, and Linford<sup>3</sup> reported for the first time what appeared to be a new disease of the potato. Although it resembled current-year symptoms of witches' broom, as described by Hungerford and Dana (8),<sup>4</sup> subsequent studies clearly differentiated between the two disorders. In December, 1927, Richards (14) ascribed this new disease to the feeding process of the tomato psyllid (*Paratrioza cockerelli* Sulc). He further reported the disease, as it appeared in Utah, as new to science and proposed the name "yellows."<sup>5</sup>

Subsequent statements have been made concerning the occurrence and the destructive nature of the disease by Linford,<sup>6</sup> Richards (15),<sup>7</sup> Metzger and Binkley,<sup>8</sup> and Binkley (1). Binkley (1) reported the disease, as it appears in the tomato, to be due to a definite virus transmitted by the psyllid nymphs. To the writers' knowledge no further report as to the nature of the disease and its relation to the tomato psyllid has appeared. It is the purpose of this paper to bring together the facts known to date regarding psyllid yellows and to report some additional experimental findings regarding the nature and cause of the trouble. The information regarding the disease is recognized definitely as being incomplete.

### OCCURRENCE OF PSYLLID YELLOWS

Psyllid yellows has scarcely a parallel in the history of phytopathology in the uniformity and rate with which it spread. The disease was first noticed by the junior author on June 12, 1927, in experimental plots at Farmington, Utah. In a preliminary survey on June 15

<sup>1</sup> Received for publication Apr. 12, 1932; issued March, 1933. Contribution from Department of Botany and Plant Pathology, Utah Agricultural Experiment Station.

<sup>2</sup> The writers wish to express appreciation for the helpful cooperation of Kathleen L. Hull, formerly of the station staff, and of William Stuart and Melvin Anderson, research students in the Department of Botany and Plant Pathology.

<sup>3</sup> RICHARDS, B. L., BLOOD, H. L., and LINFORD, M. B. DESTRUCTIVE OUTBREAK OF UNKNOWN POTATO DISEASES IN UTAH. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11:93-94. 1927. [Mimeographed.]

<sup>4</sup> Reference is made by number (italic) to Literature Cited, p. 216.

<sup>5</sup> Since the early proposal of "yellows," the name "psyllid yellows" has been employed generally by the authors and their coworkers in both correspondence and in general discussions and by the senior author in recent publications (14, 15). Binkley in 1930 also used the term "psyllid yellows" to designate the disease on tomatoes (1, *Proc. 26*, pp. 249-254). The name "psyllid yellows" seems justifiable as it is both specific and descriptive.

<sup>6</sup> LINFORD, M. B. FURTHER OBSERVATIONS ON UNKNOWN POTATO DISEASE IN UTAH. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 11:110-111. 1927. [Mimeographed.]

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<sup>7</sup> RICHARDS, B. L. PSYLLID YELLOWS (CAUSE UNDETERMINED). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 68:28-29. 1929. [Mimeographed.]

<sup>8</sup> METZGER, C. H., and BINKLEY, A. M. PSYLLID YELLOWS (CAUSE UNDETERMINED). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 68:29. 1929. [Mimeographed.]

and 16, the disease was found generally distributed in early potato fields of the Bountiful district of Davis County. Most of the fields at this early date showed 100 per cent infection, and not a single field was found which did not show the trouble to some degree. By June 29, at which time Linford commenced a more detailed survey of the disease, the early potatoes in Davis and Weber Counties were generally infested. Before the survey was concluded on September 15, Linford reported that the "disease had been found in every county of Utah where potatoes had been examined, ranging from Washington County on the south to Cache County on the north."

Table 1, copied from Linford's report,<sup>9</sup> shows the occurrence of the disease in 23 counties visited. No reports were obtained from the other six counties.

TABLE 1.—Occurrence of psyllid yellows in Utah, by counties, 1927<sup>a</sup>

County	Fields examined	Fields infested		Heaviest infestation	Average infestation
	Number	Number	Per cent	Per cent	Per cent
Cache.....	16	9	56.2	100	53.1
Carbon.....	16	15	100	100	61.1
Beaver.....	5	5	100	100	84
Boxelder.....	5	5	100	100	53.2
Davis.....	32	32	100	100	56.6
Duchesne.....	5	5	100	100	91
Emery <sup>b</sup> .....					
Garfield.....	4	4	100	100	61.2
Grand.....	1	1	100	100	100
Iron.....	21	21	100	100	83.8
Juab.....	1	1	100	10	10
Kane.....	4	4	100	100	70
Millard.....	17	9	52.9	97	24.2
Morgan <sup>c</sup> .....					
Piute.....	11	11	100	100	38.2
Salt Lake.....	24	13	54.2	100	21.7
Sanpete.....	12	12	100	100	95.8
Sevier.....	10	10	100	100	80.8
Uinta.....	6	6	100	100	68
Utah.....	9	9	100	100	44
Weber.....	11	11	100	100	74.9
Wasatch <sup>d</sup> .....					
Washington <sup>e</sup> .....					

<sup>a</sup> The figures in this table include data from many fields examined before the disease had reached its maximum spread. From the following publication: LINFORD, M. B. Op. cit., p. 98.

<sup>b</sup> Disease said to be present throughout.

<sup>c</sup> Disease reported prevalent late in season.

<sup>d</sup> Disease present.

<sup>e</sup> Crop a total failure from this disease.

Early in 1927 the malady was reported from the western slope of Colorado and was severe especially in the Fruita district of Mesa County, where 100 per cent infection occurred. According to Metzger and Binkley,<sup>10</sup> the ailment was found also in the Gunnison Valley in Delta County, in the Uncompahgre district in Montrose County, and in the Rifle district of Garfield County.

On August 1, the senior author located psyllid yellows in southern Idaho, 3.5 miles north of Pocatello. Two weeks later M. B. McKay reported the disease by letter from Idaho Falls and Twin Falls, Idaho, and from Bozeman, Mont., and H. G. McMillan in personal conference reported it from Wyoming. L. F. Nuffer by correspondence later in the summer reported a general spread of the disease in the

<sup>9</sup> LINFORD, M. B. Op. cit. See footnote 6, second reference.

<sup>10</sup> METZGER, C. H., and BINKLEY, A. M. Op. cit.

Idaho Falls district. Shapovalov<sup>11</sup> has also called attention to its presence in California.

Although the tomato psyllid (*Paratrioza cockerelli*) has been known since 1914 to be generally distributed in the Southwestern States, the writers have been unable to find any mention in literature, prior to 1927, of a disease of the potato caused by the insect; neither has reference been found to a disease of the potato similar to psyllid yellows. Comperé (2, p. 189), in 1915, states: "In Golden Gate Park, San Francisco, the solanums that were infested with psyllid were rendered worthless." He makes no further reference to the disease phase.

It appears evident from a number of authentic reports that the disease occurred previous to 1927. In 1929 Metzger and Binkley<sup>12</sup> stated:

The disease was destructive in the Fruita district of Mesa County, Colo., in 1926, and by June 18, 33 per cent of the plants were showing symptoms of a disease, evidently psyllid yellows as described by Richards in 1927.

It is quite possible also that the disease was present in the Fruita district prior to 1926. Linford<sup>13</sup> submits data which show rather conclusively that psyllid yellows was a factor in potato production in the Green River district of Utah, at least as far back as 1925. The senior author has obtained information which indicates that psyllids have figured in potato culture in Washington County, Utah, at least since 1921.

#### ECONOMIC IMPORTANCE OF PSYLLID YELLOWS

Psyllid yellows in its effect upon the plant must be ranked among the most destructive of known potato diseases. If the plant is attacked when young, prior to tuber formation, no crop results, and early death of the plant frequently ensues. When the plant is attacked during early stages of tuber formation and prior to maturation, serious injury follows, and the resulting crop is of little value. If the plant is attacked after the tubers are well formed, the tubers may sprout, giving rise to new vines (fig. 1, A, B, C), or to sprouted, knobbed, or otherwise malformed tubers, of greatly reduced market value. (Fig. 1, B, C.)

Total crop failures from this disease in individual plantings are not uncommon. In 1927 experimental plots at the Davis County Experimental Farm yielded 40 pounds of marketable tubers from an area estimated to produce from 40 to 50 bushels under normal conditions. In Bountiful, Utah, 250 pounds of marketable tubers were obtained from a 1-acre field, and many of the most successful growers in this same district lost their entire crop, leaving their fields unharvested.

During this same year the disease was so severe that total crop failures resulted in entire valleys. Linford states that in Washington County the entire crop was destroyed. This county was surveyed late in August, long after potatoes are normally harvested, but there was no harvest in 1927, so complete was the destruction.

<sup>11</sup> SHAPOVALOV, M. PSYLLID YELLOWS (CAUSE UNDETERMINED). U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 68: 29-30. 1929. [Mimeographed.].

<sup>12</sup> METZGER, C. H., and BINKLEY, A. M. Op. cit.

<sup>13</sup> LINFORD, M. B. Op. cit. See footnote 6, second reference.

Duchesne and Uintah Counties had approximately 50 per cent of a crop at the time the survey was made.

Davis and Weber County growers, who produce early potatoes chiefly, suffered the heaviest financial losses. Based on the acreage planted, it was estimated that 740 cars would have been shipped from Weber County; 110 cars were actually shipped, thus involving a loss of approximately 630 cars. Valued at \$420 a car, this amounts to \$264,600. Sixty-four cars of seed were shipped into the county at an average of 4.5 cents a pound, making a total outlay of \$92,000,

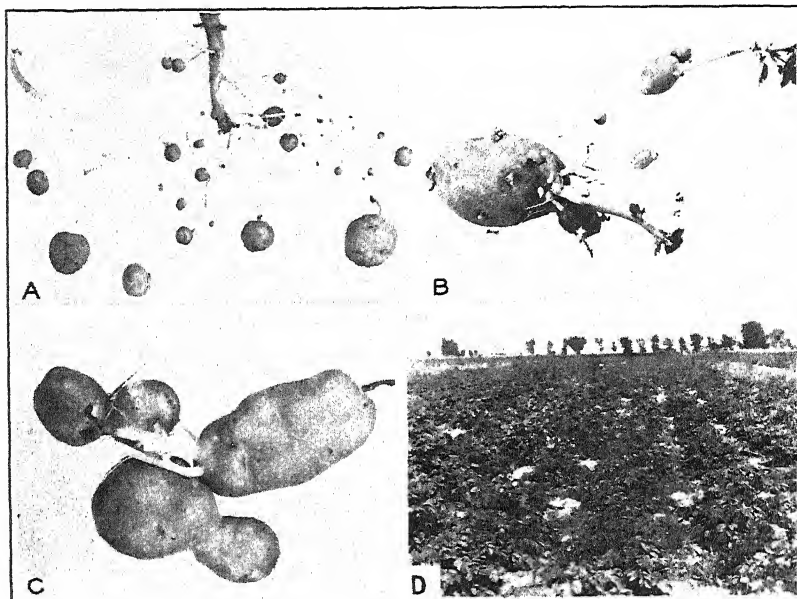


FIGURE 1.—A, Bliss Triumph affected with psyllids showing excess tuberization. One stolon has failed to terminate in tubers and has given rise to new shoots. Several shoots of this type may arise around a single infected mother plant. Note large number of secondary stolons terminating in tubers of various sizes. B, Sprouting of immature Russet Burbank tuber induced by psyllid feeding on parent plant. Practically all buds, both on the mother and the secondary tubers, have become active and have given rise either to new shoots or to a third crop of tubers. C, Sprouting of Russet Burbank tuber characteristic of this variety when parent plant is affected by psyllid nymph late in the season. Such sprouts may develop directly into tubers or may grow through the soil and give rise to second growth in the late-potato districts as in 1927. D, Five-acre field of large, vigorous, and apparently healthy Bliss Triumphs photographed October 4, 1927. These plants grew from small tubers left in the soil during the harvest of the early crop. The first crop was planted April 15 and yielded but 20 sacks per acre; on August 10 it showed 100 per cent psyllid yellows. With the exception of about 15, all plants of this 5-acre field were healthy and apparently free from psyllid yellows at the date the photograph was taken. Psyllid nymphs were present on the 15 plants showing symptoms. Seed selected from this late crop gave progeny which were entirely free from any psyllid-yellow symptoms.

or \$46,000 more than the total value of potatoes actually shipped out. While exact figures are not available, observational data indicate that Davis County suffered even more acutely than Weber County owing to more complete destruction of the crop and to the higher early market prices of potatoes. These two counties lost fully 75 per cent of their crop.

The season of 1927 was especially favorable for potato production in Utah. An average annual acre yield of 185 bushels or more might have been expected, whereas only 135 bushels were realized, entailing a loss of from 25 to 30 per cent of the crop, or approximately

\$750,000. The early crop in the State was practically a total failure. The late crop was less severely damaged although greatly reduced in market value. In certain areas little, if any, decrease in yield resulted, while in others isolated fields were seriously damaged. The malformation of the tubers of the late-maturing varieties, the Russet Burbank and the Rural, was of serious consequence. Linford estimated that from certain Russet Burbank fields in Cache Valley not more than 25 per cent of the tubers would pass as United States No. 1, and a local buyer reported that he was unable to find a single car of suitable Russet Burbank potatoes in the entire county.

Metzger and Binkley<sup>14</sup> state that in the Fruita district of Mesa County, Colo., which ships annually about 600 carloads, but 2 carloads were shipped as a result of the heavy infestation in 1927.

Losses from psyllid yellows in Utah during 1928 were greatly reduced as compared with those in 1927. Richards and his coworkers reported for 1928 that while the potato psyllid was almost coextensive with potato culture in the State, the damage was light as compared with the losses in the same areas in 1927, and that the total loss for 1928 would probably not exceed 7 per cent.

Damage to the potato crop during the year varied greatly in the different districts within the State. In Washington County, as in 1927, the destruction was again complete, and the entire crop planted between February 15 and March 10 was plowed under by June 10, except for two experimental plots. Davis and Weber Counties suffered losses between 10 and 12 per cent during the season. In a survey of the Hunter and Pleasant Green districts of Salt Lake County made during August, 1928, 72 per cent of the fields showed psyllid yellows. An average of 9.5 per cent of plants in all the fields visited showed the malady.

The disease occurred in other potato-growing areas, although there exists but little data to indicate the degree of loss. In Cache County the early garden crop was largely destroyed, and material damage was done in many of the late plantings. Sanpete and Sevier Counties were reported to have suffered severely.

Metzger and Binkley<sup>14</sup> report that psyllid yellows was not so serious in 1928 as in either of the previous seasons and that possibly 10 per cent of the acreage was infested so severely that it was plowed under and planted to other crops. The remaining acreage showed a degree of infestation varying from 0 to approximately 10 per cent.

Psyllid yellows was found in a number of areas in Utah in 1929; however, serious loss, so far as is known, was confined almost entirely to Washington County where upwards of 75 per cent of the crop was destroyed.

Psyllid yellows appeared generally throughout Utah in 1930. Reports of damage were obtained from every potato district in the State, although the damage in most areas was relatively light as compared with that of 1927. Washington County, as was the case for the last six years, again lost the major portion of its potato crop, and Utah, Davis, Weber, and Boxelder Counties sustained heavy losses to the early crop. It is significant that the early crop in these four counties, which comprises 85 per cent of the plantings, has been

<sup>14</sup> METZGER, C. H., and BINKLEY, A. M. Op. cit.

seriously reduced in yield for three of the seasons since 1927. Psyllid yellows was reported from the Twin Falls and the Idaho Falls districts in Idaho.

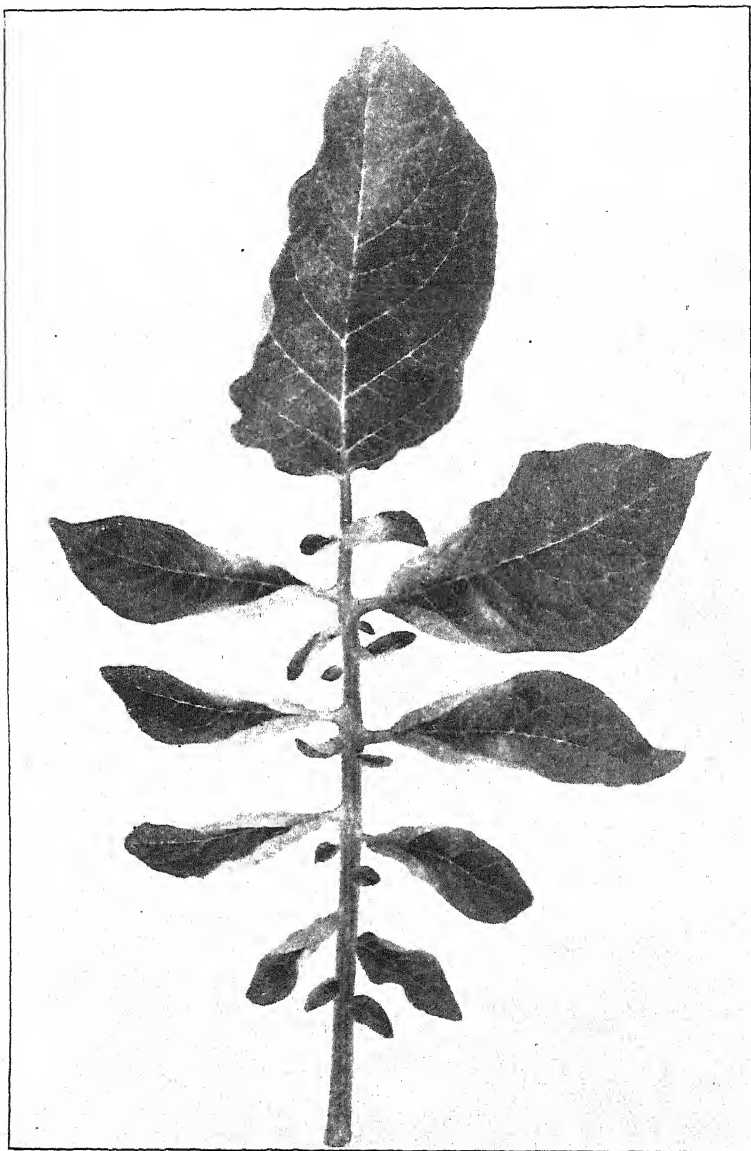


FIGURE 2.—Irish Cobbler potato leaf showing characteristic leaf symptoms of psyllid yellows. Notice the basal rolling and cupping of leaflets. These are accompanied by a characteristic yellowing and purpling, which is a typical expression of the disease on all varieties of potatoes. (Results of insect feeding under natural conditions in field)

During May and early June, 1931, psyllids appeared in great numbers in all early potato-growing areas in Utah, producing psyllid yellows in such quantities as to indicate possibilities of a general



epidemic. With the extreme heat and drought of middle and late June, however, the insects disappeared, and recovery from the disease was general. But little damage to either the early or late crop resulted. Psyllid yellows was reported severe, however, throughout the western slope of Colorado.

### SYMPTOMS OF PSYLLID YELLOWS

#### SYMPTOMS UNDER CONDITIONS ACCOMPANIED BY SUMMER-LIGHT RELATIONS

Psyllid yellows is systemic and affects the form and physiology of the entire plant. A marginal yellowing and an upward rolling or cupping of the basal portion of the smaller leaflets on the younger leaves comprise the first symptoms of diagnostic value in the field.



FIGURE 3.—A, Psyllid yellows on Irish Cobbler potato showing characteristic symptoms of the disease induced by the feeding of 200 nymphs from eggs hatched in Petri dishes; nymphs had not fed on diseased plants before transfer to the plant. Note the typical leaf cupping and particularly the peculiar upright and rigid position of young leaflets, also apical hypertrophy and axillary growth. B, Apical hypertrophy of Irish Cobbler shoot induced by confining nymphs to older leaves by means of cloth bags. Note extreme cupping of leaflets and yellowing of cupped portions

(Figs. 2 and 3, A, B.) When diseased plants are exposed to intense sunlight, this basal cupping becomes pronounced. Leaflets affected in this manner tend to curve upward over the petiole and to assume an erect position quite distinct from the normal. (Fig. 3, A.) In the Bliss Triumph and the Irish Cobbler, and to a certain extent in all varieties, the rolled portions, and frequently other aerial portions of the plant, assume a distinct reddish or purplish color which may become so pronounced in cases of severe infection as to give a purplish tint to the entire field. In the more advanced stages of the disease the older primary leaves roll upward over the midrib, become yellow, develop necrotic areas, and degenerate rapidly (fig. 4, B), and as a result a plant is produced which consists principally of secondary leaves and branches supported by the primary stems.

Promptly on the inception of the disease, the aerial shoots usually suffer a sharp delimitation in stem elongation. The nodes enlarge, and the lateral buds are stimulated into activity and may develop

into axillary tubers (fig. 3, A, B, 5) or into stocky shoots capped with a rosette of small malformed leaves, which when fully developed give the plant a compact pyramidal shape, scarcely to be recognized as that of a potato plant. (Fig. 5, A, B.) With the enlargement of the nodes and subsequent growth of the axillary buds, the subtending leaves assume a position approximately at right angles to the stem instead of the acute angle characteristic of a normal plant. (Fig. 4, D.) The apical portion of the stems, including the terminal bud

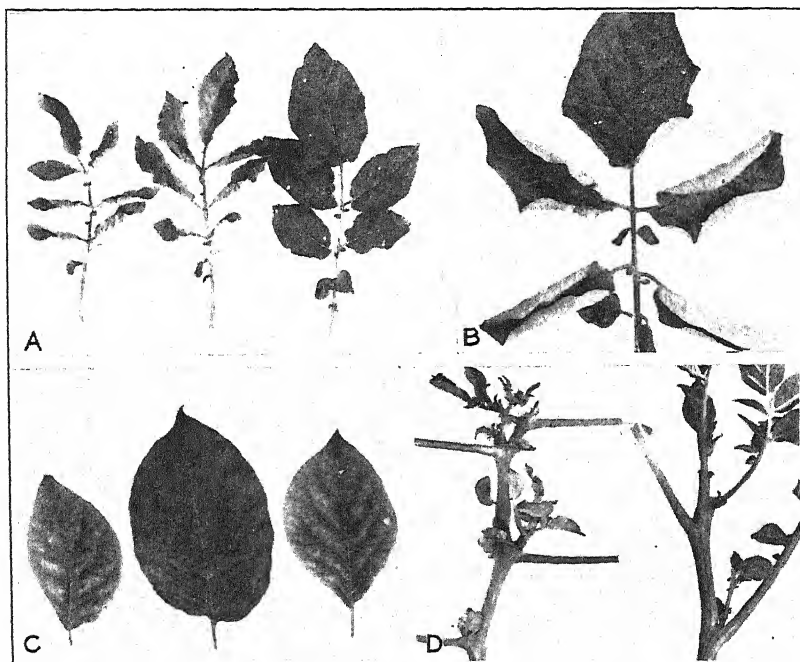


FIGURE 4.—A, Rolling and cupping of young leaflets of Russet Burbank potato very characteristic of the advanced stage of the psyllid yellows on this variety (left and center); normal leaf to the right. B, Effect of psyllid yellows on leaves which were mature at the time of the psyllid infestation. The leaves are somewhat papery and brittle in texture. Under field conditions necrosis sets in rapidly, and death soon results. This is characteristic of all varieties when affected by psyllids. C, Leaf chlorosis characteristic of psyllid yellows when affected plants are grown in the shade or during the short days of late autumn and winter. Affected leaves at left and right show distinct interveinal yellowing, also a distinct change in shape owing to an inhibition of the growth of the basal portion of leaflets, due apparently to the early destruction of chlorophyll in this region. Normal leaf in center. D, Irish Cobbler shoots showing modification of angle of leaf axis, also nodal enlargement characteristic of psyllid yellows. Left, diseased shoot (natural infection); right, healthy shoot

and frequently the first nodes and adjacent internodes, often become involved in a pronounced hypertrophy. (Fig. 3, A, B.)

If plants are attacked when young, stolon formation and tuberization are definitely suppressed, with the result that few, if any, tubers are set. When older plants are affected, the result is quite the reverse, and stolon development and tuberization are stimulated. The terminal buds of the stimulated stolons may fail to produce tubers and instead may grow directly through the soil, giving rise to a second crop of shoots adjacent to the mother plant. (Fig. 1, A.) The lateral buds along the primary stolon may develop directly into tubers (fig. 1, A), or they may give rise to secondary stolon branches which

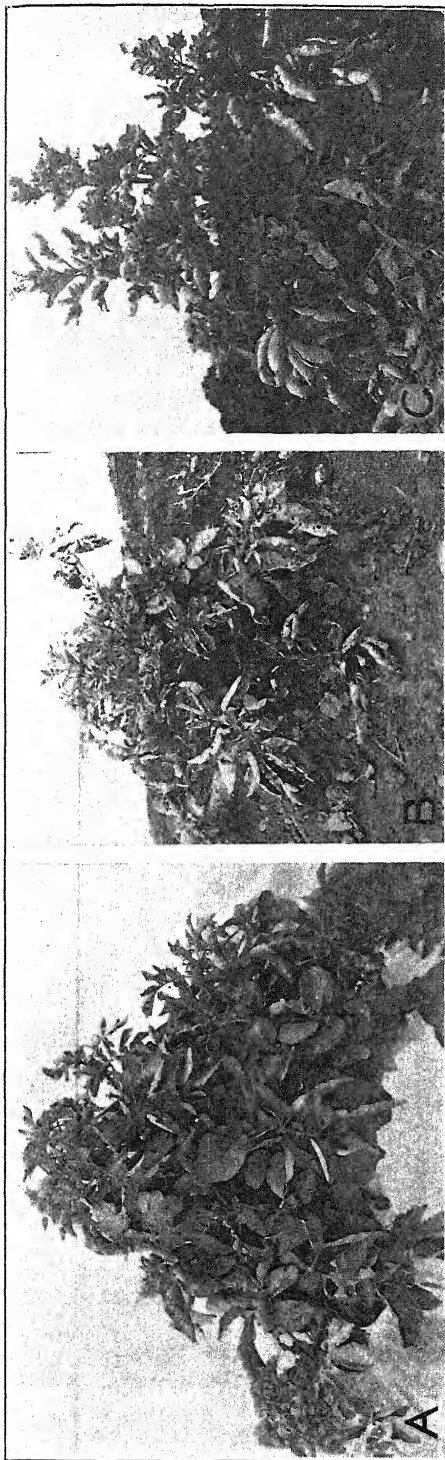


FIGURE 5.—A. Characteristic pyramidal shape assumed by the Irish Cobbler potato in the more advanced stage of psyllid yellows. This peculiar form results from inhibited linear growth and concomitant extension of the lateral secondary shoots that arise from the axis of the old primary leaves. B. Advanced stage of psyllid yellows on the Irish Cobbler. The primary leaves in advanced stage of deterioration are shown still attached to the primary stems. The lateral secondary shoots of this plant show to consist essentially of secondary shoots and shoots grown from the axis of the primary stems which have died. In the more advanced stage of the disease, the plants consist primarily of secondary leaves and shoots attached to the primary stems. C. Effect of psyllid yellows on the Early Ohio potato. Primary shoots elongate and usually of yellowish and reddish color. Note position and peculiar curling of the older leaves matured prior to psyllid infestation. This abnormal elongation is peculiar to the Early Ohio variety.

in turn may provide opportunity for tuber formation, thereby resulting in development of numerous small potatoes. (Fig. 1, A, B, D.) Such tubers seldom reach a marketable size and may sprout directly without going into a rest period. (Fig. 1, B, C.) Such sprouting may give rise to new stolons, to leafy and apparently normal shoots, or to a variety of malformed tubers. (Fig. 1, B, C.) Discarded tubers from early harvest often produce a second crop of healthy vigorous vines. (Fig. 1, D.)

Plants affected after tubers have entered the early stages of dormancy may resume growth and produce stolons and many small tubers. The more mature tubers, if still attached to the parent plant when it is attacked, may also commence growth and develop into tubers of undesirable shape and low market value. (Fig. 1, C.) This breaking of the rest period in tubers after dormancy has been established and the inhibition of its establishment in developing tubers provides one of the constant and outstanding features of psyllid yellows.

#### SYMPTOMS UNDER CONDITIONS OF REDUCED LIGHT INTENSITY

The types, vigor, and sequence of symptomatological expression vary so greatly under both the intensity and the duration of light that symptoms characteristic of the open field are of little value in judging results under protected conditions in the greenhouse or under conditions of relatively dense shade. This fact was first suggested from observations made during the winter of 1927. Subsequent study, however, has provided a basic symptomatology for the study of psyllid yellows under shaded conditions and during the late fall, winter, and early spring months, and under cloth cages during the summer months. In fact, this symptomatology appears quite as reliable as that under natural conditions in the field and has been used as a criterion of judgment for all work conducted under cloth or under winter greenhouse conditions.

With decreased light intensity and duration, basal cupping and coloration do not occur uniformly. Instead, the basal lobes of the young leaflets turn distinctly yellow and are inhibited in growth, giving rise to linear leaflets quite distinct from those of the normal leaf. (Figs. 3, A; 4, C.) Yellowing finally appears in the interveinal portions of the affected leaflet, giving a general leaf chlorosis, seldom seen under field conditions. (Fig. 4, C.) A similar type of interveinal yellowing develops progressively from the younger to the older leaves, advancing slowly in later stages and showing a typical interveinal chlorosis in the most advanced stages of the trouble. The necrotic areas characteristic of the late stages of leaf deterioration in the open field have not as yet been observed under the more protected conditions. On the whole, the rate at which the plant succumbs to the disease under conditions of reduced light intensity and duration is much slower than that found in the open field; diseased plants under such conditions may survive quite as long as and sometimes longer than the healthy individuals.

The degree and type of bud activity both below and above the ground appear to be affected but little with varying light conditions.

## NATURE AND CAUSE OF PSYLLID YELLOWS

## RELATION OF THE PSYLLID TO THE DISEASE

During the early survey studies in June, 1927, Linford<sup>15</sup> first noted a small scalelike insect on plants showing symptoms of psyllid yellows. Subsequent studies showed a high correlation between the occurrence of the insect, which was later determined to be *Paratrioza cockerelli* by the Bureau of Entomology (fig. 6, A, B), and the characteristic symptoms of the disease. Survey studies throughout 19 counties in Utah showed this parallelism to be complete. Even in the most remote and isolated fields the disease and the insect were found constantly associated. In August, 1927, M. B. McKay, in a personal letter, reported the disease from Montana and southern Idaho, and in all cases the disease was associated with the insect. A

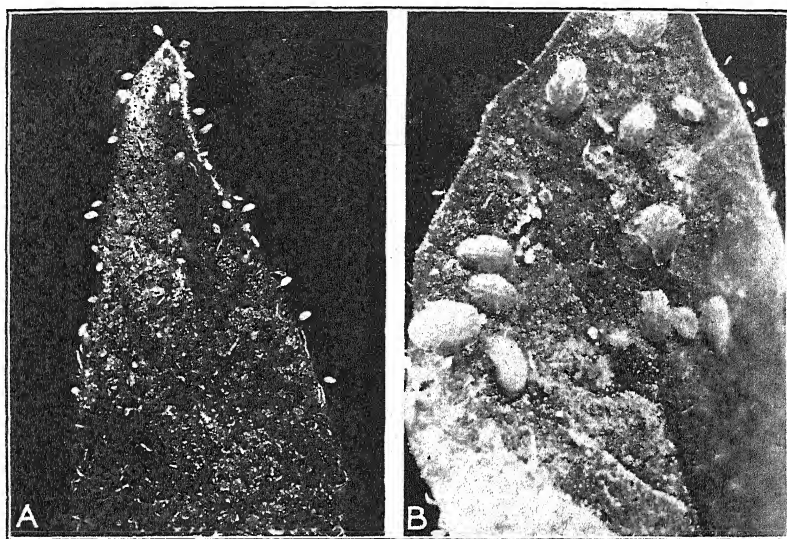


FIGURE 6.—A, Eggs of *Paratrioza cockerelli* Sule on young potato leaflet.  $\times 9$ . B, Nymphs of *P. cockerelli* feeding on young potato leaflet. Note eggs on leaf margin and scattered irregularly over the surface of the leaf.  $\times 9$

similar report was obtained from H. G. McMillan for Wyoming and Colorado. Such observations indicated rather clearly that the tomato psyllid was definitely concerned in the etiology of the disease.

On July 15, psyllids were taken from Davis County and placed on 50 plants in the pathological garden at Logan and covered with gauze insect cages. All plants developed the disease. Later, however, the disease spread throughout the  $\frac{1}{2}$ -acre experimental plot, finally involving 100 per cent of the exposed plants. Forty plants in this plot, however, had been grown under insect cages and were kept covered until October 15, and 39 of the 40 remained entirely free from the disease. During the season a triangular rent was made in one of the cages, through which parts of the covered plant protruded. This plant became infested with insects and developed a clear case of psyllid yellows.

<sup>15</sup> LINFORD, M. B. Op. cit. (See footnote 6, first reference.)

Five experiments to test the etiological relation of *Paratrioza cockerelli* were made during September and October, 1927. On September 5, 40 psyllids were confined on each of three plants grown in the greenhouse under celluloid cages. Similarly, caged plants free from insects were held as controls. By September 9, one of the plants with insects had developed definite basal leaf rolling, a second plant had developed symptoms by September 18, and a third by October 2.

On September 9, 30 nymphs were transferred to each of 8 plants grown under celluloid cages. Three plants covered with cages but free from the insects were used as controls. By September 28, all plants on which the insects fed had developed advanced stages of the psyllid yellows disease. The controls remained normal.

On September 20, 30 nymphs were placed on each of 3 plants under celluloid cages; 2 plants were kept for controls. By September 30, 10 days later, 2 of the plants had exhibited marginal yellowing and basal rolling, and the third had developed the disease by October 10. The 2 control plants remained disease-free until discarded on November 1. Another series was started September 21 in which 30 insects were transferred to each of 7 plants. Four plants were held as controls. By October 10, the 7 insect-infested plants had developed clear-cut symptoms of the disease. The controls at this date were free from symptoms and insects. Later 1 of the check plants was found infested with nymphs and subsequently developed the disease.

In the fifth series plants were started in the greenhouse without cages. These plants were grown in 8 inches of soil on the greenhouse bench. All plants were in a vigorous growing condition on September 23, at which time the transfer of insects was made. Twenty plants distributed uniformly throughout the bed were employed and nymphs were carefully placed on 1, 2, or 3 leaves of each of the plants. The nymphs were confined to the leaves with gauze bags in such a way as to maintain complete isolation of other parts of the inoculated plant and of the control plants. Thirty insects were used to each plant; 51 plants were left as controls. Of the 20 to which insects were transferred, 3 showed marginal yellowing and basal leaf rolling within nine days. On October 10, 15 plants exhibited basal rolling as well as the characteristic yellowing and reddening. By November 1, when the experiment was terminated the plants had developed the disease and exhibited the various symptoms characterizing psyllid yellows in the field. The 51 controls remained disease-free. Nineteen diseased and nineteen healthy hills were selected and the tubers dug and weighed to determine the effect of the disease on tuberization. The data are summarized in Table 2.

TABLE 2.—Effect of psyllid feeding on tuberization of Irish Cobbler potatoes in preliminary experiments

Condition of plant	Hills	Tubers	Average tubers per hill	Average total weight per hill	Average weight per tuber
	Number	Number	Number	Grams	Grams
Diseased.....	19	317	16.6	75.5	4.5
Healthy.....	19	79	4.2	92.5	22.0

In these preliminary tests all phases of the disease as found in the field were produced under controlled experimental conditions, following an incubation period of six to nine days. The results justify the conclusion that the disease in some way is induced during the feeding process of nymphs of *Paratrioza cockerelli*.

#### RELATION OF NYMPHAL FEEDING TO THE DISEASE

##### RELATION OF THE NUMBER OF NYMPHS TO SYMPTOM EXPRESSION

In the preliminary tests typical symptoms of psyllid yellows were produced consistently with as few as 30 active nymphs. In late autumn what were considered characteristic symptoms of the disease failed to develop in the greenhouse on plants carrying as many as 60 to 75 nymphs. The following March, however, the progeny of these insects which had failed in the winter to induce psyllid yellows again consistently induced the disease. At first the cause for this apparent lack of potency on the part of the insect was not clear. In the course of the experiments, however, it became apparent that both the intensity and the duration of sunlight in some way either influenced the insect in its feeding or determined the type of symptom expression.

During 1928 and 1929 attempts were made to determine more accurately the quantitative relation between nymphal feeding and the disease. On August 11, three series of experiments were started in the field under gauze cages, using 10, 15, and 30 nymphs per plant. The time of feeding varied from 3 to 15 days, after which time the insects were killed by fumigation. The results are given in Table 3. All plants on which psyllids fed showed the early symptoms within 9 to 15 days after the insects commenced feeding. A study of the subsequent development of the disease on these plants was rendered valueless because of the general psyllid infestation of the field.

TABLE 3.—Relation between number of psyllid nymphs and the length of feeding period and the first symptoms of psyllid yellows under cages in open field

[Period of feeding, 3 to 15 days, commenced August 11, 1928]

Series No.	Plants	Nymphs per plant	Length of feeding period	Plants diseased	Series No.	Plants	Nymphs per plant	Length of feeding period	Plants diseased
	Number	Number	Days	Number		Number	Number	Days	Number
1	4	30	3	4	3	2	10	5	2
	4	30	3	4		2	10	7	2
	4	30	7	4		4	10	10	4
	4	30	10	4		2	10	15	2
	4	30	15	4					
2	4	15	5	4					
	4	15	10	4					

Between April 22 and July 25, 1929, four separate series of tests were completed, using from 1 to 9 nymphs per plant. In these tests nymphs failed to produce psyllid yellows, although comparable numbers during the past three years have occasionally been noted to induce the disease.

Additional data were obtained in four sets of experiments conducted under greenhouse conditions between March 19 and June 25, 1929. The numbers and the distribution of the nymphs, together with a summary of results, are given in Table 4.

TABLE 4.—*Summary of four series of experiments, showing relation between numbers and distribution of psyllid nymphs and the symptom expression of psyllid yellows on the Irish Cobbler potato*

[Feeding period, March 19 to April 12, 1929; data taken 41 days after insect transfer, 12 plants used in each experiment]

Distribution of nymphs	Psyllids per plant	Plants diseased	Plants remaining healthy	Distribution of nymphs	Psyllids per plant	Plants diseased	Plants remaining healthy
Nymphs placed on one leaf-----	300	11	1	Nymphs distributed equally on three leaves—	30	4	8
	150	11	1		15	0	12
	75	7	5		6	1	11
	30	3	9	Continued.	300	12	0
	15	3	9		150	12	0
Nymphs distributed equally on three leaves.	6	0	12		75	10	2
	300	12	0		30	7	5
	150	10	2		15	1	11
	75	9	3		6	0	12

Results of all the various experiments indicate that under greenhouse conditions and during the shorter days of late fall or early spring the number of nymphs feeding, and possibly the distribution of the insect on the plant, are vital factors in the production of psyllid-yellows symptoms. Much additional work under controlled environmental conditions must be done to determine more accurately this quantitative relation of nymphs to the occurrence of the disease.

#### RELATION OF NYMPHAL FEEDING PERIOD TO THE PRODUCTION OF EARLY SYMPTOMS OF THE DISEASE

Experiments were started on March 6, 1930, to determine the shortest period of insect feeding necessary to produce psyllid yellows. In the experiment, 200 nymphs (100 on each of two leaves) were confined on each of 20 healthy plants. Insects were removed at intervals as follows: Plants 1-5, after 2 days; plants 6-10, after 3 days; plants 11-15, after 4 days. Insects on plants 16-20 were allowed to feed for the entire period of 36 days. Ten plants, Nos. 21-30, were kept free from insects and remained free from the disease throughout the entire experiment. This experiment was repeated with essentially the same results. The results are recorded in Table 5.

Results indicate that at least three days' feeding is necessary to produce the first definite symptoms of psyllid yellows. Four days' feeding gave a more vigorous expression of the early symptoms and resulted in a more permanent effect, although with a 4-day period the disease did not progress far beyond the early manifestation.

This failure of symptoms to appear as a result of fewer than three days' feeding was not occasioned by any peculiar masking effect of the environment, as the early appearance of the disease resulted when the same number of insects fed for longer periods, and a complete expression of symptoms was produced by nymphs feeding continuously for a period of 36 days.



TABLE 5.—*Relation between short periods of nymph feeding and the production of early or first symptoms of psyllid yellows in the Irish cobbler potato*

[Feeding time 2, 3, 4, and 36 days; 200 insects used on each plant]

Plant No.	Feeding period	Condition of plant at removal of nymphs	Condition of plant 10 days after feeding commenced	Condition of plant 36 days after the beginning of nymph feeding
	<i>Days</i>			
1.....	2	No symptoms.	Normal.....	Large plant apparently normal.
2.....	2	do.....	do.....	Very slight marginal yellowing, otherwise normal.
3.....	2	do.....	do.....	Do.
4.....	2	do.....	Mere trace of marginal yellowing.	Slight basal yellowing and restriction, otherwise normal.
5.....	2	do.....	Normal.....	Slight basal yellowing, otherwise normal.
6.....	3	do.....	Slight interveinal yellowing.	Basal, marginal, and interveinal yellowing distinct early symptoms.
7.....	3	do.....	Normal.....	Normal except slight yellowing of young leaves.
8.....	3	do.....	Slight trace of yellowing on young leaflets.	Slight yellowing of 3 leaves, otherwise normal.
9.....	3	do.....	Normal.....	Young leaflets yellow and some interveinal yellowing could be considered type of early symptoms.
10.....	3	do.....	Slight marginal yellowing.	Distinct basal yellowing on a number of younger leaves.
11.....	4	do.....	Normal.....	Do.
12, 13, 14.....	4	Symptoms	Early symptoms of disease; basal, marginal, and interveinal yellowing.	Distinct basal, marginal, and interveinal yellowing on younger and older leaves, showing that feeding had some effect on subsequent growth.
15.....	4	do.....	Marginal, basal, and interveinal yellowing.	
16, 17, 18, 19, 20.....	36	do.....	Marginal, basal, and interveinal yellowing; all symptoms exhibited.	Full expression of symptoms of disease above and below ground.
20-30.....	0	Healthy...	Healthy.....	Healthy, with occasional plants showing slight yellowing of younger leaflets.

## RELATION OF NYMPHAL FEEDING PERIOD TO THE SEQUENCE AND DEGREE OF SYMPTOM EXPRESSION

During the earlier greenhouse and field studies instances were observed frequently which indicated the existence of a definite relation between the length of the feeding period and the final degree of disease expression. In many cases, especially under greenhouse conditions, apparent recovery was noted, and in general the accumulated evidence suggested that continued feeding of nymphs was necessary to produce the full expression of psyllid yellows.

On February 23, 1930, two series of experiments were started to determine more accurately the relation of the feeding period to the early appearance and to the continued development of symptoms of psyllid yellows. Twenty plants, exclusive of controls, were used in each of the two series. In series 1, 200 nymphs (100 on each of two leaves) were confined by means of gauze bags to each of 20 healthy plants. The insects were then removed from the plant by clipping off the infested leaf as follows: Plants 6–10, after 12 days' feeding; plants 11–15, after 16 days, and plants 16–20, after 26 days' feeding. Plants 1–5, inclusive, were fed upon for 48 days.

Series 2 was set up in the same manner as series 1. Plants 1–5, inclusive, were fed upon for a period of 50 days, or during the entire time the experiments were run. Insects were removed as follows:

Plants 6-15, after 9 days; plants 16-20, after 14 days. Eight insect-free plants were used as controls for series 1 and nine plants for series 2. All plants in both series were grown openly in the greenhouse bench in 8 inches of soil. A summarized statement of series 1 and 2 is recorded in Table 6. (Fig. 7, A, 1-5.)

TABLE 6.—*Summary of results of experimental series 1 and 2 in which 200 psyllid nymphs were fed on Irish Cobbler potato plants for various periods longer than the incubation period*

[See text for details and also Figure 6A, 1-5]

SERIES 1

Length of feeding	Plants involved	Average height	Average number of leaves per plant	Average length of leaves	Average tubers per hill	Average weight of tubers per hill	Average weight per tuber	General expression of symptoms at the end of the experiments, which lasted 50 days
Days	Number	Inches	Number	Inches	Number	Ounces	Grams	
0	5	16.0	10.3	10.3	4.5	6.7	42.2	Normal.
12	5	15.2	11.6	11	S. S	5.8	18.7	Some apparently normal; others slightly yellowed; some axillary growth.
16	5	14.2	13	9.9	S. S	4.05	13.0	Distinct interveinal yellowing; abundant axillary growth.
26	5	13.1	12	9.9	11.4	4.02	10.4	Clearly diseased; yellowing of younger and older leaves.
50	5	11.3	12	S. 7	14.0	1.6	3.2	Severe disease of all plants; yellowing, reddening, and cupping of apical leaves; apical and axillary hypertrophy.

SERIES 2

0	8	14.3	-----	9.8	4.8	5.6	23.1	Normal.
9	10	13.7	-----	9.08	9.0	5.4	17.0	Some apparently normal; others slightly yellowed; some axillary growth.
14	5	13.3	-----	8.3	8.4	4.6	15.5	Distinct interveinal yellowing; abundant axillary growth.
50	5	12.8	-----	9.5	13.4	2.3	4.8	Severe disease of all plants; yellowing, reddening, and cupping of apical leaves; apical and axillary hypertrophy.

Most of the infested plants in the two series showed slight marginal and interveinal yellowing by the sixth day. All plants showed unmistakable symptoms at the end of the tenth day. Many plants exhibited marked rolling and cupping of the smaller and younger leaflets. Basal, marginal, and interveinal yellowing was pronounced on all plants from which insects were removed on the twelfth day in series 1 and by the ninth day in series 2. A number of these plants also showed basal leaf cupping and reddening of younger leaves. More advanced symptoms, such as leaf rolling, pronounced interveinal yellowing, slight nodal enlargements, and beginning axillary growth, were evident at the end of the sixteenth and twenty-sixth days.

In all cases, on the removal of the insects, the progress of development of the symptoms appeared to cease rather abruptly. All red and purple coloring that had developed during the feeding period on affected leaves completely disappeared. Leaf rolling or cupping ceased, and the rolled leaves uniformly unrolled and assumed normal

position and shape. A definite tendency toward recovery was noted also in both marginal and interveinal yellowing. Where but slight yellowing occurred prior to insect removal, recovery was apparently complete; however, when the yellowing was well advanced, the

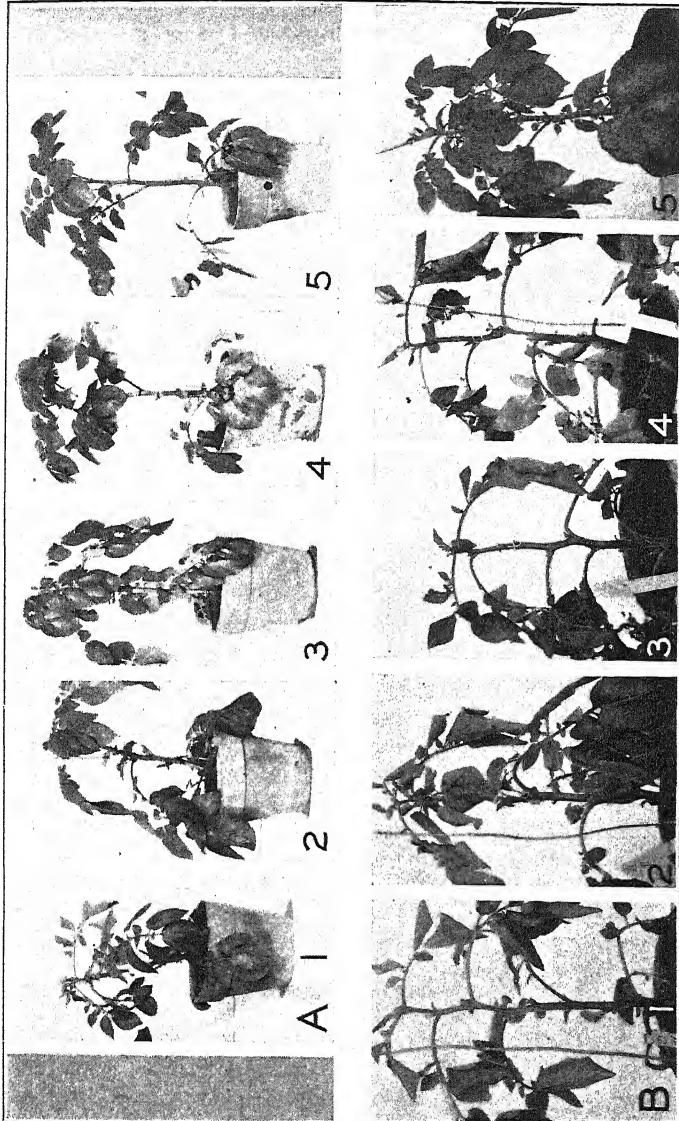


FIGURE 7.—A, Irish Cobbler potatoes on which psyllid nymphs have fed for varying periods. For data see page 200 and Tables 10 and 11. The feeding periods were as follows: Plant No. 1, 50 days; plant No. 2, 26 days; plant No. 3, 16 days; plant No. 4, 12 days; plant No. 5, no feeding (control). B, Psyllid yellows induced in Irish Cobbler plants by nymphs from eggs hatched in Petri dishes on normal potato leaves. Plants Nos. 1, 2, and 3 are representative of separate series on which nymphs from different broods, kept free from all diseased plants, fed. Plant No. 4, injured by nymphs from diseased potato plants. Plant No. 5, control, kept free from all sources of infection.

normal green color was seldom regained. Plants fed upon for fewer than 16 days assumed practically normal linear growth and in many cases equaled the unaffected plants. Subsequently, axillary and apical growth was of a normal green color and of apparently normal texture. Certain physiological processes, however, were so disturbed

during the feeding that the plants did not completely recover from the effects of the disease. This is clearly shown in the relative height of plants (fig. 7, A), number of tubers per hill, average weight of tubers per hill, and average weight per tuber. (Table 6.) In all cases, a greater number of tubers with a decreased total hill yield and decrease in tuber weight resulted with increased length of feeding. All symptoms characteristic of the disease developed prominently in plants on which the insects were fed for the total 50 days. (Fig. 7 A, 1.)

Results indicate clearly that the symptom complex characteristic of psyllid yellows is entirely contingent upon the feeding process of nymphs of *Paratrioza cockerelli* and further that a complete expression of symptoms in their characteristic sequence and intensity, under greenhouse conditions, is entirely dependent on the continued feeding of the nymphs.

#### RELATION OF ADULT FEEDING TO THE DISEASE

Studies with nymphs have given no clue as to the exact effect of adult feeding on symptom expression. Two sets of experiments were set up July 16, 1929, in which adult psyllids were allowed to feed for a period of 28 days on healthy Irish Cobbler plants in the open field. In the first series, 50 adults were fed on each of 10 plants. These were confined in groups of 10 on each of 5 different leaves of the plant, by means of small spring cages. In the second series, 75 insects were fed on each of 10 plants, 15 in each spring cage on each of the 5 separate leaves. Five of the insect-infested plants of each series were then placed under insect-proof cages and kept covered for the entire time of the experiment. The other five plants of each series were left uncovered and exposed to the natural weather conditions in the field. Cages with insects were shifted to new feeding areas on the leaf every third day and the eggs laid during the period destroyed. All insects lost through death or escape were promptly replaced by additional insects from the insectary. No indication of the disease developed from adult feeding either during or subsequent to the 28-day period.

Five additional series of experiments were started on August 22 in the greenhouse to determine whether adults in numbers greater than those used in the earlier field studies could induce yellows. In these experiments the insects were confined to the plants in large celluloid cages under which the plants had been grown, thus allowing feeding over the entire plant surface. To insure against nymph feeding, the adults were removed at intervals of four days and all eggs destroyed. In this process, adults were removed from the cages by suction guns and placed in small vials. The eggs were then crushed and the original number of insects replaced. All adults used in the experiment were obtained from diseased plants in the insectary.

In series 1, 100 adults were placed on each of 10 plants on August 22 and allowed to feed until September 16, a total of 25 days. At the end of the feeding period, none of the plants showed symptoms of psyllid yellows. Eggs laid during the last 4-day period of insect feeding, September 12 to 16, were allowed to hatch on 6 of the 10 plants used in the experiment. All 6 of these plants showed typical symptoms within 9 to 12 days after the hatching of the eggs. The other 4 plants, kept free from nymphs, did not develop psyllid yellows after the removal of the adults.

Series 2 was a duplication of series 1 except that 150 instead of 100 adults were used. Eight of the ten plants remained free from symptoms for 28 days, at which time the experiment was terminated. Two plants showed unmistakable symptoms of disease. When examined, however, both diseased plants were found to carry a number of psyllid nymphs, hatched from eggs which were overlooked in the experiment. All adults were removed from the 10 plants at the end of 28 days. As in series 1, the eggs laid during the last 4 days of the experiment were allowed to hatch on 6 plants, all of which developed psyllid yellows within 8 to 12 days after the nymphs commenced feeding.

Series 3, 4, and 5 differed from 1 and 2 only in the number of adults used. In series 3, 200 adults were allowed to feed on each of 5 plants; in series 4 and 5, 500 and 1,000 adult insects, respectively, were placed on each of 3 plants. After 28 days' feeding, none of the 11 plants in the three series showed the slightest symptom of disease. Plants fed upon by these larger numbers, however, were evidently stunted in growth, and when the numerous nymphs from eggs laid during the last four days of the experiment were allowed to feed, 7 of the plants were killed.

The five experiments on adult feeding were carefully checked, (1) by placing 50 nymphs on 10 healthy plants and allowing them to feed during the 28 days, all of them developing psyllid yellows, and (2) by placing 7 plants under cages not exposed to insect feeding but treated as if eggs were being crushed at the same period as eggs were crushed in the experiments. Except for apparent stunting and occasional leaf mutilation, these latter plants showed no indication of treatment. A final check was provided by growing 10 plants under cages free from insects and untouched. The check plants remained free from disease during the 28 days of the experiment and grew somewhat larger than did those exposed to insects.

Results of the five series on adult feeding are given in Table 7.

TABLE 7.—Results of five series of experiments in which various numbers of adult psyllids were fed for 25 days on healthy Irish Cobbler potato plants

Experiment No.	Insects used per plant	Stage of insect	Plants used	Diseased plants	Healthy plants
1.....	100	Adult.....	10	0	10
2.....	150	do.....	10	" 2	8
3.....	200	do.....	5	0	5
4.....	500	do.....	3	0	3
5.....	1,000	do.....	3	0	3
Control.....	60	Nymph.....	20	20	0

<sup>a</sup> As explained in the text, these 2 plants showing unmistakable symptoms were found to have a number of nymphs feeding on them.

#### RELATION OF NYMPHS TO INFECTIVE PRINCIPLE

The failure of adult psyllids to induce the disease at once raises the question as to the origin or source of the substance injected by nymphs which produces the pathological symptoms characteristic of psyllid yellows in the potato. An attempt was made to separate the nymphs from this substance by removing eggs from the leaves of potato

plants to healthy disease-free leaves in Petri dishes where they were allowed to hatch. The resulting nymphs were then transferred to healthy plants on which they were allowed to develop into adults and finally to lay eggs. From these eggs three separate broods of nymphs (A, B, and C) were obtained and were placed on healthy potato plants in the greenhouse. Two hundred nymphs were placed on each plant used in the experiment. Two plants were used for insects from brood A, 3 for brood B, 4 for brood C. Nymphs were allowed to feed for 27 days. Eight plants were held free from insects as controls, and additional plants were inoculated, each with 200 nymphs from diseased potato plants. All nine plants fed on by the nymphs from broods A, B, and C developed uniformly symptoms entirely characteristic of psyllid yellows and in a more severe form than that produced by nymphs from diseased potato plants. The results are recorded in Table 8.

TABLE 8.—*Results from the feeding of psyllid nymphs which were obtained from eggs hatched in Petri dishes on disease-free potato leaves*

[Feeding period, 27 days, March 25 to April 22, 1929]

Source of insects	Plants employed	Insects per plant	Diseased plants	Healthy plants	Incubation period	Degree of severity of disease
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Days</i>	
Brood A.....	2	200	2	0	6	Severe expression.
Brood B.....	3	200	3	0	6	Do.
Brood C.....	4	200	4	0	6-8	Do.
From diseased plants.....	8	200	8	0	6-8	Mild to severe; only 2 plants equalled in severity the plants fed upon by insects, broods A, B, and C.
Insect-free (control).....	8	0	0	8	-----	

### TRANSMISSION STUDIES

During 1928 and 1929 various standard methods used for the artificial transfer of virus diseases were employed in attempts to transmit psyllid yellows from diseased to healthy potato and tomato plants. Large numbers of plants in different stages of development were used as sources of inoculum and for inoculation. All these attempts gave negative results. Binkley (1, v. 26) also reports negative results in his attempts to transmit artificially the disease in the tomato. In preliminary experiments grafting has also proved ineffective as a means of transmission. To date, the nymph of *Paratrioza cockerelli* appears to be the only known means by which psyllid yellows can be transmitted from diseased to healthy plants, if such transfer actually takes place, or by which the disease is induced directly into healthy plants. Further work is now in progress to test all known methods of artificial transmission.

Observational and experimental evidence further indicates that psyllid yellows is not transmitted through the tuber from diseased parents to the progeny under field or greenhouse conditions in Utah. Young plants from attached tubers produced by diseased plants or from tubers detached from the mother plants show no symptoms of the disease; however, both may develop the disease with equal readi-

ness when fed upon by psyllid nymphs. It is also quite possible that young attached shoots may in time develop the disease through the mother plant, when the latter is fed upon continually by nymphs, although this relation has not been clearly established. In the field shown in Figure 1, D, only 18 plants were found to exhibit distinct psyllid symptoms on October 4, 1927, when the photograph was taken. Nymphs were found actively feeding on all of these. Three bushels of tubers selected from this same crop and planted in 1928 produced vigorous plants entirely free from any signs of the disease.

Because of the vigor of the plants, the owner selected seed from this same stock for his 1929 planting. Although in 1928 the plants were infested with the psyllids later in the season, the plants in 1929 again appeared entirely free from the disease and remained so throughout the season. Other observations similar to the foregoing were made during 1927 to 1930, inclusive, and plantings made for the purpose of determining this relation gave similar results. In 1929, 15 fields of second-crop potatoes from discarded infested tubers were observed to show no symptoms of psyllid yellows until fed upon by psyllid nymphs later in the season. Under no observed conditions have plants, grown from tubers produced by infected parents and kept free from insects, shown symptoms of psyllid yellows either in the greenhouse or in the field.

## EPIDEMIOLOGY OF PSYLLID YELLOWS

### GENERAL NATURE

The sudden appearance of psyllid yellows in 1927 provides an interesting problem for analysis. According to data already presented, the disease appeared rather suddenly in the early crop throughout Utah, the western slope of Colorado, southern Idaho, and local areas in Montana and Wyoming. This apparently sudden and extensive distribution was no less startling than the degree of uniformity with which the disease occurred. In Utah few, if any, fields in the infected areas escaped the disease, and in certain localities, particularly those growing early potatoes, infection was so complete that scarcely an unaffected plant could be found. Isolated fields in canyons many miles removed from the main valley centers of potato culture became infected before the end of the season. A similar situation existed throughout the western slope of Colorado where the disease almost completely destroyed the potato crop in many districts.

### FACTORS INVOLVED

The sudden appearance and widespread and uniform distribution of psyllid yellows in 1927 are explainable only by the presence of some prolific and rapidly disseminated etiological factor, such as is found in the tomato psyllid. The distribution of *Paratrioza cockerelli* prior to 1927, the nature of the insect, that is, its natural means of dissemination, rate of development, longevity and degree of fecundity, insect parasitology, and the number and distribution of host plants are, therefore, vital factors. The various environmental factors, such as temperature, humidity, and air movement enter into the problem chiefly as a result of their influence on the fecundity and dissemination of the insect.

## DISTRIBUTION OF THE INSECT PRIOR TO 1927

The suddenness with which psyllid yellows occurred and the novelty of the disease in the infested areas during 1927 suggests an early migration of *Paratrioza cockerelli* from a few local areas in which the insect normally survived into the various parts of Utah, Idaho, Montana, Wyoming, and parts of Colorado. On the other hand, it is quite possible that the insect had previously occupied these infected areas but had occurred in such small numbers as to entirely escape notice, especially as an etiological factor; thus, with a disturbance of some peculiar balance, the involvement of the entire region of the 1927 epidemic was made possible.

Survey studies subsequent to 1927 have established the fact that *Paratrioza cockerelli* had been present and responsible for psyllid yellows during 1925 and 1926 in the Green River and Washington County districts in Utah and in certain districts in Colorado. It is conceivable that the insect might have spread from these various areas throughout the range occupied in 1927. The more general distributions of the insect reported by Crawford (4) in 1914 appears to furnish the more plausible explanation for the 1927 epidemic of the disease. This writer gives the distribution of the species of *Paratrioza cockerelli* as covering the entire southwestern part of the United States and reports the specific localities in which the insect has been found as follows: Boulder, Rocky Ford, and Canon City, Colo.; Milford and Park City, Utah; Tucson and Fort Yuma, Ariz.; Madagascar Mountains, N. Mex.; Claremont, El Centro, San Luis Obispo, Argus Mountains, Alameda, and Death Valley, Calif. The occurrence in Milford and Park City, Utah, is especially interesting, and the further fact that the Utah station entomologists have observed *Paratrioza cockerelli* in the infested areas in previous years suggests the distinct possibility that the insect might have been more generally distributed than had been supposed. The ability of the insect to overwinter in those areas to which it spread in 1927 and its early appearance in Davis County, Utah, apparently bear out this possibility.

## NATURE OF THE INSECT

Crawford (3, 4), Ferris (7), and Lehman (10) describe *Paratrioza cockerelli* as a small homopterous insect of the family Cheridae with a small, active clear-winged adult stage, varying in size from 1.3 to 1.8 mm in length and from 0.39 to 1 mm in width. This small adult is extremely active; when disturbed, it springs quickly into the air by means of its powerful hind legs. The springing habit of the insect, assisted by flight and by the wind, undoubtedly functions effectively in the distribution of the species and consequently in the spread of the disease at least within relatively localized areas. The insect is so minute that when once in the air it might readily be transported considerable distances by the air currents. It is conceivable that the combination of these factors may result in the transportation of the insect over long distances.

Observations as to the longevity of the adult vary. Compere (2) noted that *Paratrioza cockerelli* adults lived for three months in captivity. Knowlton and Janes (9) gave 64 days as the longest life period for an adult male and 189 days for an adult female, with an average life of 25.22 days for males and 34.4 days for females. The



writers found the life of the female to vary from a few hours to 60 days, with an average longevity under greenhouse conditions of 45 days. The long life of the insect may result in overlapping of broods, which fact, coupled with a long oviposition period, aids greatly in increasing the number of affected individuals.

Compere (2) observed that egg laying commenced three days after copulation, although he did not state the length of the preoviposition period. Knowlton and Janes (9) reported a preoviposition period varying from 5 to 25 days, with an average of 10.1 days from emergence of adult female until the first eggs were produced. Similar results to those of Knowlton were obtained by the writers. It will be noted from Table 9 that the preoviposition period for the 10 females varied from 4 to 20 days, with an average of 9.7 days between emergence and the production of the first eggs. Compere (2) stated that three adults in captivity laid eggs for a period of 3 days. Knowlton and Janes (9), on the other hand, gave a much longer period of egg laying, varying from a few days to a maximum of 179 days. They found the average oviposition period of 58 females to be 21.45 days. For 10 insects observed under greenhouse conditions, the writers found the oviposition period to vary from 20 to 53 days, with an average of 35.2 days. (Table 9.)

TABLE 9.—*Egg-laying history of 10 adult females of Paratrioza cockerelli (Sulc) under greenhouse conditions*

[One pair of adults was confined to leaves of potatoes by means of spring cages; at the end of each 24 hours the cages with insects were removed to a new leaf area and eggs laid during the 24 hours counted]

Adult No.	Pre-egg-laying period of adult	Length of egg-laying period	Total eggs laid	Adult No.	Pre-egg-laying period of adult	Length of egg-laying period	Total eggs laid
	<i>Days</i>	<i>Days</i>	<i>Number</i>		<i>Days</i>	<i>Days</i>	<i>Number</i>
1.....	5	20	430	8.....	20	35	553
2.....	6	21	458	9.....	17	31	497
3.....	11	33	851	10.....	5	36	641
4.....	16	28	504				
5.....	4	53	1,460	Minimum.....	4	20	430
6.....	6	46	979	Maximum.....	20	53	1,460
7.....	5	49	829	Average.....	9.7	35.2	720

In the shortest oviposition period of 20 days, as shown in Table 9, the insect laid 430 eggs, while the female with an oviposition period of 53 days deposited 1,460 eggs. Also, as shown in Table 9, the 10 insects averaged 720 eggs. Knowlton and Janes (9) report a total of 19,833 eggs from 60 females, with an average of 330.55 eggs for each of the 60 females. These writers also record a maximum of 1,352 eggs for a single female, laid during a period of 179 days.

Knowlton and Janes (9, p. 285) record a high percentage hatchability of psyllid eggs. Of 9,615 eggs observed, 7,989, or approximately 83<sup>16</sup> per cent, hatched, giving rise to living nymphs. The large number of eggs, their hatchability, and the extended period during which the female psyllid may lay eggs undoubtedly plays an important part in the epidemiology of psyllid yellows.

*Paratrioza cockerelli* passes through an incomplete metamorphosis with the usual stages of adult, egg, and nymph. The time required

<sup>16</sup> This should be 83 per cent.

for completion of the life cycle has been variously estimated and has been shown to depend to quite an extent upon the influence of environment on the development of the insect during the various stages of growth. Compere (2) reports that approximately 45 days were evidently required to complete the life cycle under the conditions imposed in his experiments. He further states that the broods "are continuous throughout the year." Lehman (10) gives 25 days as the time necessary for the life cycle of the insect. Knowlton and Janes (9, p. 286), in laboratory studies with a much larger number of insects than observed by Compere, give the time for development of the various instars shown in Table 10.

TABLE 10.—Time of development of various instars of *Paratrioza cockerelli* as given by Knowlton and Janes

Instar	Nymphs observed	Average days required	Instar	Nymphs observed	Average days required
First.....	252	2.76	Fourth.....	151	2.72
Second.....	186	2.44	Fifth.....	133	4.87
Third.....	158	2.49			

Knowlton and Janes (9) state that the total time for nymphal development for some 800 nymphs observed varied with conditions from 12 to 21 days, with an average of 16 days. These data correspond essentially with the observations of the present writers. Judging from the data supplied by Knowlton, the life cycle of *Paratrioza cockerelli* from egg laying to adult emergence may be completed within from 25 to 37 days, depending on the conditions to which the insect is exposed. These data are significant in view of the fact that nymphs have been observed under the rather vigorous climate at Logan, Utah, as early as May 4, and in an active state as late as November 2. In view of these data, it appears possible that from three to five broods may develop during a single season. Consequently, the progeny from a single female adult under favorable conditions may number into the millions in a single season. In view of such fecundity and length of life favorable ecological conditions for overwintering and propagation may initiate an epidemic any season.

#### PARASITOLOGY OF INSECT

Little is known of the parasites of *Paratrioza cockerelli*, although the writers have observed that such do occur and undoubtedly play an important part in the survival of the species. Whether the absence of psyllid parasites played a significant part in the epidemic of 1927 and in the persistence of the insect during 1928-29 and 1930-31, there is no way of determining. However, as the variation in the physical environment appears inadequate to explain the epidemic of 1927, it would seem that the abundance and sudden appearance of the insect during the particular year might be adequately explained by a serious disturbance of the biological balance which ordinarily keeps the insect in check. The parasitology of *Paratrioza cockerelli* remains as a problem for further study.

## NUMBER AND DISTRIBUTION OF HOST PLANTS

The food plants of *Paratrioza cockerelli* are undoubtedly important in facilitating the distribution of the insect. The degree of influence which these food plants exert, however, is still problematic, although the number of plants on which the insects have been found is significant. Crawford (4, p. 72) lists food plants as follows: "Pepper (*Capsicum annuum*), tomato (*Solanum nigrum*), potato (*Solanum tuberosum*), \* \* \* spruce (*Picea* sp.) pine (*Pinus monophylla*), alfalfa (*Medicago sativa*). List (11, 12) reports psyllids as being abundant on the cultivated tomato in Colorado.

Ferris (7) observed adults and nymphs from California on tobacco (*Nicotiana tabacum*), and Compere (2) stated that the insects on which he made observation in the Golden Gate district of California were found on the Jerusalem cherry (*Solanum capsicastrum*). Van Duzee (17) listed the insects on members of the following genera: *Capsicum*, *Solanum*, *Purshia*, *Picea*, *Pinus*, and *Medicago*. Essig (5, p. 438) in 1917 added *Datura* sp. and *Solanum nigrum* to the list of host plants. The writers have found *Paratrioza cockerelli* nymphs and adults feeding abundantly on tomato (*Lycopersicum esculentum*), groundcherry (*Physalis longifolia*), matrimony-vine (*Lycium vulgare*), and on all varieties of the potato (*Solanum tuberosum*) grown in Utah during the past three years. In the greenhouse the nymphs have been found to feed on tobacco.

The common groundcherry appears to be a most favorable host of *Paratrioza cockerelli* in Utah, second possibly only to the potato in importance. In 1927, this plant was universally infested with psyllids throughout the State, and even in isolated areas miles from potato fields it was found to support large numbers of both nymphs and adult psyllids. The general distribution of this *Physalis* species in Utah undoubtedly facilitated the dissemination of *P. cockerelli* in 1927 and figured as an important factor in the epidemic of psyllid yellows in that year, as well as in the general epidemiology of the disease.

## DISCUSSION

Accumulated evidence clearly indicates that *Paratrioza cockerelli* in some definite way is involved in the etiological complex of psyllid yellows. Exactly in what manner the insect produces the disease, however, has not been revealed, although some facts are sufficiently suggestive as to merit definite consideration. The total absence of visible disturbances at the point of insect feeding, together with the systemic nature of psyllid yellows and the few insects necessary to induce the disease, precludes mechanical injury or food extraction as possible elements in disease production. On the other hand, these basic relations considered in connection with other features of the disease suggest more definitely that the disease is produced either by a virus, transferred from plant to plant, or by some toxic substance injected into the plant tissue during the feeding process of *P. cockerelli* nymphs. Facts have been obtained which support both possibilities.

The more critical studies have revealed facts which are difficult to fit into the virus theory. The abrupt cessation of symptom development with the removal of the insect followed by an apparent

uniform tendency to recover from the disease, is especially pertinent in this connection. These facts, when taken together with the development of apparently healthy organs from axillary buds and the absence of tuber transmission of the disease, renders acceptance of the virus concept particularly difficult. In this connection it is also necessary to recall that the adult psyllid is incapable of producing psyllid yellows after feeding on diseased plants, and that the infective principle, whatever its nature, is probably inseparable from the psyllid nymphs, which alone are capable of producing the disease. The foregoing, however, must be considered in view of the fact that Shapovalov (16) reports tuber transmission of psyllid yellows and that Binkley (1) claims to have separated that which he considers as a virus from the nymph and has unhesitatingly designated the disease as of virus origin. On these two points the meager data presented by these workers raise a serious question as to the justification for their conclusion. Whether or not the substance injected into the potato plant by *Paratrioza cockerelli* is a virus remains a question for future research.

To the writers the explanations that appear most plausible, especially in view of a number of the foregoing facts, is that *Paratrioza cockerelli* during its feeding processes injects into the plant tissues some toxic substance which quickly becomes systemic and possibly produces the exaggerated responses characteristic of the disease by its interference in some way with the carbohydrate metabolism of the plant.

It is interesting in this connection to note that Monteith and Hallowell (13) report a condition involving the feeding of leaf hoppers on legumes which resemble closely the etiological complex and symptomatological responses involved in psyllid yellows. They suggest that the pathological symptoms involved are probably the result of some "chemical or enzymatic toxin," secreted by the leaf hoppers.

Should the toxin theory of psyllid yellows prove correct, it would appear that this disease, also the whitetop of alfalfa and other legumes, the hopperburn of potatoes, and possibly other diseases, constitute a group of plant maladies that may truly be designated, based on their peculiar etiology, as insect diseases.

#### SUMMARY

Psyllid yellows as a disease of the potato first came to the attention of plant pathologists in 1927, although there is evidence that the trouble has existed in certain isolated areas in Utah for a number of years prior to this date.

The disease may develop suddenly over a vast area in any one season and is capable of extensive and frequently complete destruction in both the early and the late potato crop.

Studies since 1927 have shown the dangerous nature of psyllid yellows and indicate that in certain districts it is a perennial menace even to the extent of eliminating the potato as a crop. This condition exists particularly in Washington County in southern Utah, and also in the early potato-growing areas of Davis and Weber Counties in the northern part of the State. A similar condition is reported also for the Fruita section in the Grand Junction district of Colorado (western slope).

In survey studies the tomato psyllid (*Paratrioza cockerelli* Sule) has been found constantly associated with psyllid yellows of the potato, and experiments have shown that the disease is in some way induced during the feeding processes of the nymphs of this insect.

Under conditions especially favorable for symptom expression as few as three to five nymphs might occasionally produce psyllid yellows, although uniformity of appearance and full expression of symptoms seldom result with fewer than 15 to 30 actively feeding nymphs.

The adult form of *Paratrioza cockerelli* in numbers up to 1,000 per potato plant appear incapable of producing psyllid yellows symptoms on the plant in the field or in the greenhouse.

The symptomatology of psyllid yellows varies greatly with the number of insects feeding, the length of feeding period, and the intensity and duration of light exposure during the time of feeding.

Psyllid-yellows symptoms under conditions of unmodified sunlight consist of yellowing, basal leaf rolling and purpling of the younger leaves, yellowing and rolling of older leaves, nodal enlargement, increased axillary angle, aerial tubers and shoots, frequent rosetting, various apical growths, and distortion, excess tuberization, and inhibition of rest period. Under conditions of decreased exposure and intensity of light, basal, marginal, and interveinal yellowing becomes a constant feature of the disease.

Progress in symptomatological expression is stopped abruptly when insects are removed at intervals of 12, 16, and 26 days after feeding has commenced, indicating that the full expression of symptoms of psyllid yellows results only when nymphs of *Paratrioza cockerelli* are allowed to feed continuously on the tissues of the infested plant. A tendency toward recovery results when time of feeding in the greenhouse is less than 16 days. Recovery in the field has also been observed. So far as is known, *P. cockerelli* is the only factor capable of producing psyllid yellows in the potato and in related plants.

Attempts to transmit psyllid yellows from diseased to healthy plants have failed. Under Utah conditions the disease does not appear to be transmitted from diseased plants to the following generation through the tuber.

The size, motility, prolific fecundity, longevity, and long oviposition period of the female are important factors in the rate of dissemination of the insect. These factors, when considered in connection with the apparent general distribution of the insect, provide, in part, at least, an explanation of the sudden widespread epidemic of psyllid yellows in 1927.

In preliminary tests nymphs of *Paratrioza cockerelli* were not separated from the infective principle by hatching eggs on healthy potato leaves in Petri dishes. In fact, nymphs so hatched produce a more vigorous symptom response on healthy Irish Cobblers than psyllid nymphs of the same age grown on infected potato plants.

The true nature of the infective principle injected into potato plants by *Paratrioza cockerelli* at present remains unknown. Available facts, however, question somewhat the virus theory of the disease and suggest the possible existence of some toxic substance which is produced in some way during the feeding process of the psyllid nymphs. Additional facts will be necessary before final conclusions can be drawn as to the true etiology of psyllid yellows.

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# CHROMOSOMES IN GOSSYPIUM AND RELATED GENERA<sup>1</sup>

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## INTRODUCTION

An investigation of the chromosomes in the Malvaceae, particularly in *Gossypium*, was undertaken because of the repeated failures of cotton breeders in their attempts to hybridize such cottons as Garo Hill (*Gossypium cernuum* Tod.) with any of the varieties of upland cotton (*G. hirsutum* L.).

In 1923 a study was made of the chromosomes in a few representative species of *Gossypium* growing in the greenhouses at Washington, D. C. Among these were found some species with 13 and some with 26 as their haploid chromosome number. Neither number corresponded with that found earlier by Cannon (4)<sup>3</sup> or Balls (1). Simultaneously with two other investigators, Denham (5, 6), of England, and Nikoljeva as reported by Zaitzev (15, 16), of Russia, the writer found that cultivated varieties of *Gossypium* fall into two classes, those with 13 chromosomes and those with 26 chromosomes. This classification corresponds with the taxonomic separation of *Gossypium* species into the Old World, or Asiatic, and the New World, or American, groups.

## MATERIAL AND METHODS

The present investigation has been confined to pollen mother cells during the reduction phases. Young buds were selected and the staminal column removed and put at once into Bouin's, Carnoy's, or chromo-acetic (1:1:100) killing solution, embedded, sectioned, and stained. It was soon found that very satisfactory preparations of pollen mother cells in which the chromosomes were dividing could be made from fresh material stained in aceto-carmin fluid. This method was used whenever growing material was near enough to the laboratory for smear preparations to be made promptly after the young buds were picked. When this was impossible, the anthers were put immediately into killing fluid; later they were embedded or, if Carnoy's killing reagent had been used, it was often possible to make aceto-carmin preparations that served for chromosome studies.

## CHROMOSOMES IN THE ASIATIC GROUP OF COTTONS

The term "Asiatic cottons," as now understood, designates a distinct group of *Gossypium* species having the haploid chromosome number 13, characteristic of all cottons of Asiatic origin thus far examined.

<sup>1</sup> Received for publication May 25, 1932; issued March, 1933.

<sup>2</sup> The writer acknowledges the helpful cooperation of the Divisions of Cotton, Rubber, and Other Tropical Plants and of Egyptian Cotton Breeding, Bureau of Plant Industry. The Asiatic  $\times$  American F<sub>1</sub> plant used in this study was obtained from J. B. Norton and resulted from an accidental cross in the cotton-breeding nursery at Hartsville, S. C.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 226.

Table 1 shows the *Gossypium* species and varieties, most of which are endemic to the Old World, in which various investigators have found the basic chromosome number 13.

TABLE 1.—*Cottons (Gossypium spp.) in which the haploid chromosome number 13 was found by various investigators*

Denham (8)	Zaitzev (15, 16)	Banerji (2)	Longley
<i>G. arboreum</i> (3 varieties). <i>G. cernuum</i> . <i>G. indicum</i> (2 varieties). <i>G. neglectum</i> .	<i>G. arboreum</i> . <i>G. herbaceum</i> . <i>G. indicum</i> . <i>G. obtusifolium</i> .	<i>G. arboreum</i> (2 varieties). <i>G. cernuum</i> . <i>G. herbaceum</i> (10 varieties). <i>G. indicum</i> (5 varieties). <i>G. neglectum</i> (9 varieties). <i>G. obtusifolium</i> (2 varieties). <i>G. stocksii</i> .	<i>G. arboreum</i> (C. B. <sup>a</sup> 620). <i>G. cernuum</i> (C. B. 422). <i>G. davidsoni</i> <sup>b</sup> (C. B. 101). <i>G. harknessii</i> (C. B. 862). <i>G. herbaceum</i> (C. B. 254-b). <i>G. indicum</i> (C. B. 430, 472, 517, 726, 727, 743; S. P. I. <sup>c</sup> 12139, 12141, 12142, 12146, 12149). <i>G. intermedium</i> Tod. (C. B. 619). <i>G. neglectum</i> (C. B. 506). <i>G. obtusifolium</i> (C. B. 435). <i>G. sturtii</i> <sup>d</sup> (C. B. 564).

<sup>a</sup> C. B. refers to accession number of the Division of Cotton, Rubber, and Other Tropical Plants.

<sup>b</sup> Harland (8) reports 13 as the haploid chromosome number for this species.

<sup>c</sup> S. P. I. refers to serial designation number of the Division of Foreign Plant Introduction.

<sup>d</sup> Harland (8) reports 26 as the haploid chromosome number for this species.

Pollen mother cells of *Gossypium* are large and are characterized by a dense granular zone in the cytoplasm just outside the nucleus. This zone has been noted by all the earlier investigators of the Malvaceae and, according to Beal (3), it is of importance in the formation of spindle fiber.

During the reduction phases the chromosomes appear unusually small in comparison with the large mother cell. They are frequently well separated, however, and stand out sharply in the comparatively clear nuclear region. Figure 1, A to E, shows the chromosomes in a few representatives of the Asiatic group of *Gossypium*. A is a diakinesis from *Gossypium herbaceum*. The chromosomes at this stage show variations in shape and their bivalent nature is quite apparent. The two parts of one chromosome at the left have been separated by a slight pressure on the cell. In Figure 2, A and B, are two photomicrographs of the first-division metaphase showing the chromosomes on the plate in *G. cernuum*, variety Garo Hill. Figure 1, B, shows an early first-division metaphase from *G. indicum* Lam. The chromosomes are more compact than in Figure 1, A, and are approaching the plate stage. Figure 1, C, shows a late first-division metaphase of *G. obtusifolium* Roxb. Some of the chromosomes have divided, others have not. Figure 1, D, shows an early first-division telophase in *G. indicum*. The two groups of 13 chromosomes were from different planes and the drawing shows them approaching the two poles of the mitotic spindle. Figure 1, E, is a second-division metaphase of *G. neglectum* Tod., showing clearly the 13 chromosomes.

There are three other cottons that have the same chromosome number as the Asiatic group: *Gossypium davidsoni* Watt., of Lower California and Sonora; *G. harknessii* Brandeg., of Lower California; and *G. sturtii* F. v. M., of Australia. Harland (8) reports 13 as the chromosome number for *G. davidsoni*, but gives 26 as the number for *G. sturtii*. Figure 3, A, shows a typical diakinesis of *G. davidsoni*. The number for *G. sturtii* has been determined by the writer on two different



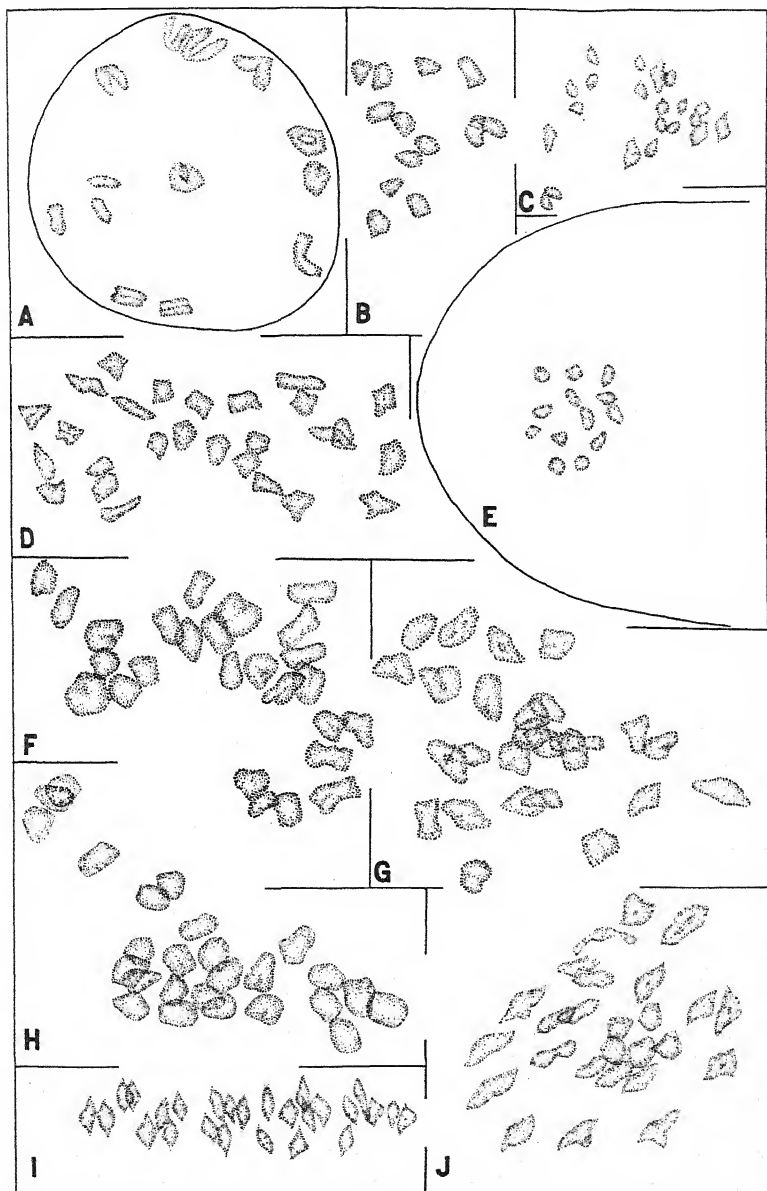


FIGURE 1.—Chromosomes from pollen mother cells of *Gossypium* ( $\times 1,500$ ): A, Diakinesis of *G. herbaceum*; B, early first-division metaphase of *G. indicum*; C, late first-division metaphase of *G. obtusifolium*; D, early first-division telophase of *G. indicum*; E, second-division metaphase of *G. neglectum*; F, late diakinesis of *G. hirsutum* (Acala); G, late diakinesis of *G. barbadense* (Pima); H, late diakinesis of *G. barbadense* (sea island); I, metaphase of a wild cotton from Florida; J, early metaphase of *G. hirsutum*  $\times$  *G. barbadense* (Tuxtla  $\times$  sea island)

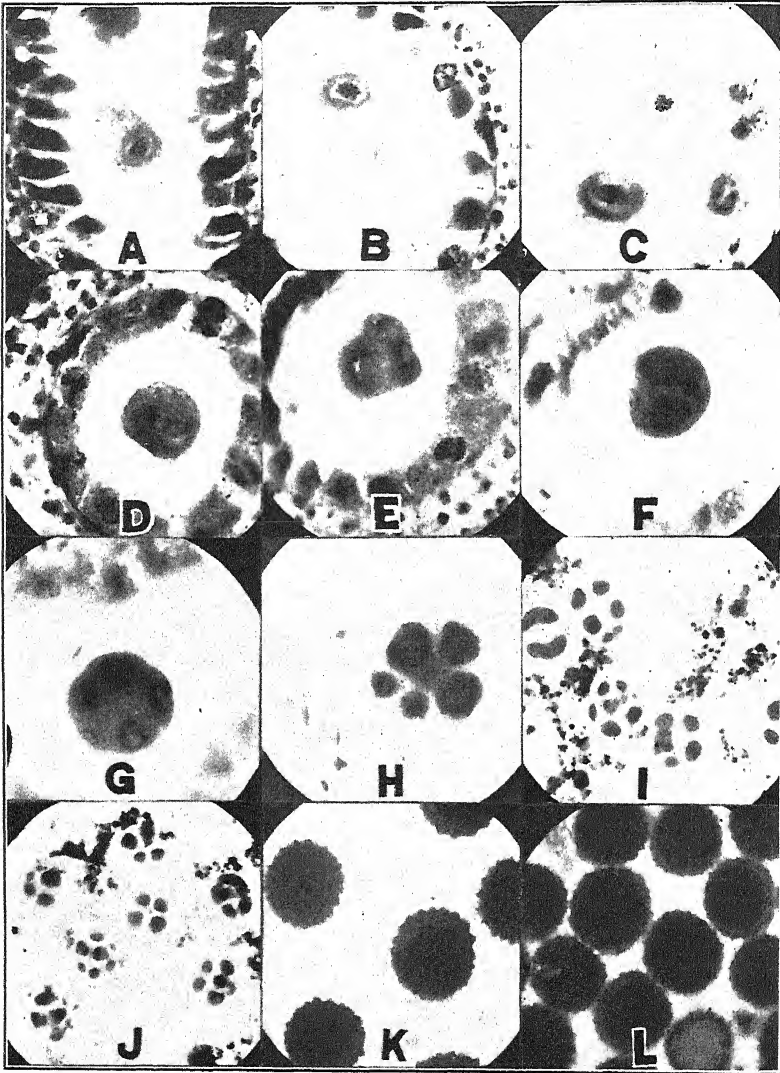


FIGURE 2.—Photomicrographs showing development of pollen in *Gossypium* (× 500): A and B, First division metaphase of *G. cernuum* (Garro Hill); C, first-division metaphase of *G. hopi* Lew-  
 ton; D, first-division metaphase of *F1* Asiatic × American hybrid; E, interkinesis of *F1* Asiatic  
 × American hybrid; F, second-division metaphase of *F1* Asiatic × American hybrid; G, late  
 telophase of Asiatic × American hybrid; H, irregular tetrad from Asiatic × American hybrid; I  
 and J, irregular tetrads from *F2* Holdon × Pima (upland × Egyptian) hybrid; K, pollen from  
 sea island (*G. barbadense*); L, pollen from Acala (*G. hirsutum*)

occasions, and in the early phases of the first-reduction division seems to be unquestionably 13.

For *Gossypium stocksii* Mast., a wild species of northwestern India, Youngman and Pande (14) found the haploid number 13. This finding has been confirmed recently by Banerji (2).

For *Gossypium arboreum* L.  $\times$  *G. neglectum* and *G. cernuum*  $\times$  *G. indicum*, two hybrids between species of the Asiatic group, Denham (6) found the haploid number 13. He makes no reference to abnormalities in chromosome behavior.

#### CHROMOSOMES IN THE AMERICAN GROUP OF COTTONS

The American group of cottons is best represented by *Gossypium hirsutum*, typical of the Mexican subgroup, and by *G. barbadense*

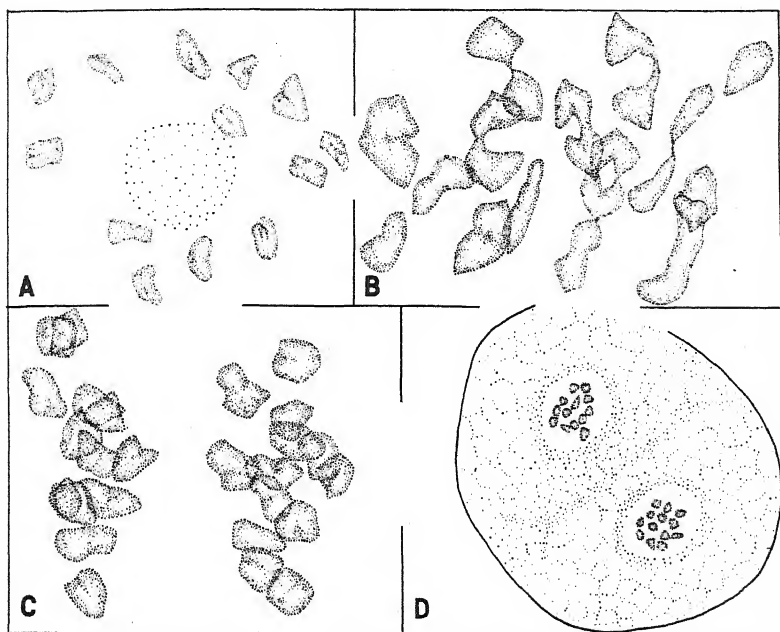


FIGURE 3.—Chromosomes from pollen mother cells of *Gossypium* and related genera ( $\times 1,500$ ): A, Diakinesis of *G. davidsoni*; B, first-division metaphase of *Shantzia garckeana*; C, first-division anaphase of *S. garckeana*; D, second-division metaphase of *Thurberia thespesioides*

L. and *G. peruvianum* Cav., typical of the South American subgroup. These subgroups apparently include all cottons endemic to North America and South America, except the wild species *G. davidsoni* and *G. harknessi*, of Lower California, and *G. klotzschianum* Anderss., of the Galápagos Islands.

Table 2 shows the *Gossypium* species and varieties of the Mexican and South American subgroups in which various investigators have found the chromosome number 26.

A late diakinesis of Acala cotton (*Gossypium hirsutum*) is shown in Figure 1, F; in Figure 1, G and H, are shown similar phases of two cultivated forms of *G. barbadense*, Pima and sea island, respectively. In all three cases the 26 chromosomes are shown just before collecting on the plate. Figure 1, I, shows a side view of the first-division meta-

phase plate from a cotton growing wild in Florida. By pressure on the cell the 26 chromosomes were spread out and separated in order to show their characteristic shapes just prior to division. In Figure 2, C, is a photomicrograph showing the chromosomes on the plate in *G. hopi*.

TABLE 2.—Cottons (*Gossypium* spp.) in which the haploid chromosome number 26 was found by various investigators

Denham (5, 6)	Zaitzev (15, 16)	Banerji (2)	Beal (3)	Longley
<i>G. barbadense</i> (15 varieties), <sup>a</sup> <i>G. hirsutum</i> (5 varieties).	<i>G. barbadense</i> . <i>G. brasiliense</i> . <i>G. fruticosum</i> . <i>G. hirsutum</i> . <i>G. lanceolatum</i> . <i>G. mexicanum</i> . <i>G. microcarpum</i> . <i>G. mustelianum</i> . <i>G. palmerii</i> . <i>G. peruvianum</i> . <i>G. punctatum</i> . <i>G. purpurascens</i> . <i>G. schottii</i> . <i>G. vitifolium</i> .	<i>G. hirsutum</i> (3 varieties).	<i>G. barbadense</i> (2 varieties). <i>G. hirsutum</i> (3 varieties).	<i>G. barbadense</i> (C. B. 331-c, 363, sea island and Pima). <i>G. brasiliense</i> (C. B. 535). <i>G. hirsutum</i> (Acala). <i>G. hopi</i> (2 varieties). <i>G. punctatum</i> (C. B. 437).

<sup>a</sup> Balls (1) gives 28 as the chromosome number of this species.

In a study of 26 American cottons no exception has been found to the characteristic haploid chromosome number, 26.

Cannon (4) was the first to investigate the chromosomes in *Gossypium*. In his study of a hybrid between *G. barbadense* and *G. hirsutum*, representing a cross between two distinct American types, he found 20 as the haploid chromosome number. Figure 1, J, shows the chromosomes in a similar cross, between the varieties Tuxtla (*G. hirsutum*) and sea island (*G. barbadense*). This hybrid had 26 as its haploid chromosome number, and shows no marked abnormalities in chromosome behavior.

#### CHROMOSOMES IN A CROSS BETWEEN A PLANT OF THE ASIATIC GROUP AND ONE OF THE AMERICAN GROUP

Recently Zaitzev (15, 16) and Desai (7) have described successful crosses made between plants of the Asiatic and American groups of cotton. Their attempts to hybridize plants of the Asiatic and American groups gave a large percentage of failures, but a few  $F_1$  plants were obtained. The pollen of these plants proved to be sterile. An Asiatic  $\times$  American  $F_1$  plant studied by the writer produced abundant flowers, but like those of Zaitzev and Desai, has been perfectly sterile.

Pollen mother tissue was sufficiently abundant in the anthers to make possible a detailed examination of the chromosomes during the reduction phases. Figure 4, C, shows a characteristic first-division metaphase with 14 bivalent and 11 univalent chromosomes. The number of paired chromosomes was variable; only when they could be distinguished from the univalent chromosomes was it possible to get accurate counts. Figure 2, D, shows a photomicrograph of a typical first-division spindle, with the bivalents on the plate and the univalents scattered on the spindle.

In Figure 2, E, is a photomicrograph taken at interkinesis, showing two major nuclei and a minor nucleus. This photograph also gives a fair idea of the granular zone in the cytoplasm that surrounds the nuclei.

A view of the second-division metaphase in a pollen mother cell is shown in Figure 4, D. One plate has 20 full-sized chromosomes and 2 halves of a univalent chromosome that divided in the previous division. The other plate shows 17 large chromosomes and 2 halves of a divided univalent. A much more irregular and more typical second-division metaphase is shown in Figure 4, E. Six spindles have been formed. The chromosome number in each spindle varies from a single univalent to 13 univalents and 6 halves of divided univalents.

Figure 2, F, shows a photomicrograph of a pollen mother cell with two major spindles and a small spindle on the margin of the cell.

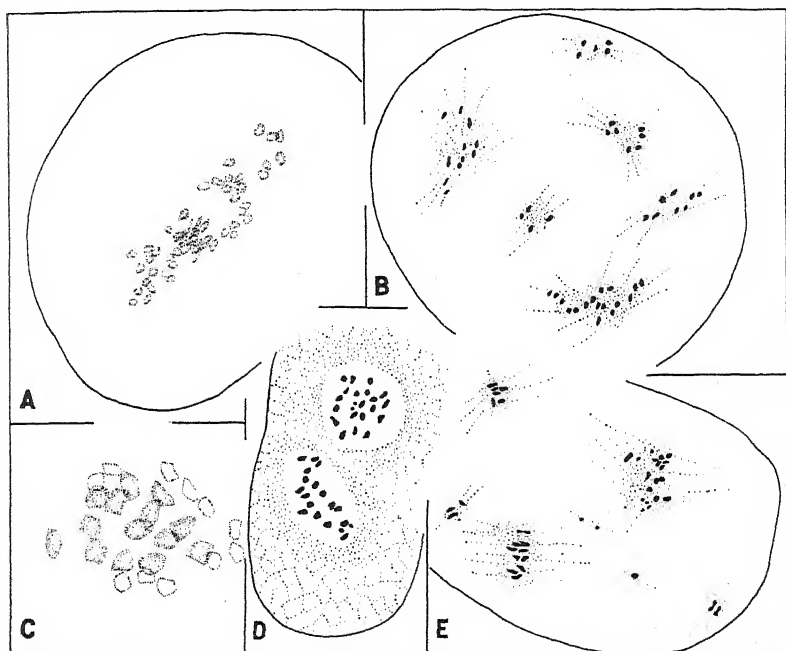


FIGURE 4.—Chromosomes from pollen mother cells of *Gossypium* hybrids: A, First-division metaphase of an  $F_2$  plant of Holdon  $\times$  Pima (upland  $\times$  Egyptian); B, second-division metaphase, showing 6 spindles of an  $F_2$  plant of Holdon  $\times$  Pima; C, diakinesis of an Asiatic  $\times$  American hybrid, showing bivalents and univalents in outline; D, second-division metaphase of an Asiatic  $\times$  American hybrid, showing unequal numbers of chromosomes on the two plates; E, second-division metaphase of an Asiatic  $\times$  American hybrid, showing 2 major and 5 minor spindles

Figure 2, G, shows the late telophase of a pollen mother cell just prior to cell division.

Chromosome distribution during the reduction phases of the hybrid was generally irregular and led to the production of abundant abnormal pollen tetrads and sterile pollen.

Nakatomi (10) has recently described the cytological behavior of the chromosomes during pollen formation in an American  $\times$  Asiatic  $F_1$  hybrid. In this cross, although it differed in some details from the reciprocal cross described by the writer, Nakatomi found the pollen tetrads similar and the mature pollen variable in size and apparently sterile.

## CHROMOSOMES IN RELATED GENERA

A study was likewise made of the chromosomes in certain genera closely allied to *Gossypium*.

For *Thurberia thespesioides* A. Gray, the haploid chromosome number proved to be 13, the same as that of the Old World species of *Gossypium*. Figure 3, D, shows a typical pollen mother cell from this species, in which 13 chromosomes show distinctly in the two metaphase plates.

*Shantzia garckeana* Lewton, another plant related to *Gossypium*, was grown in the greenhouses at Washington, D. C., from seeds collected by H. L. Shantz in Central Africa. This species also has 13 as its haploid chromosome number. Figure 3, B and C, shows the chromosomes of *Shantzia* during a late first-division metaphase in which 2 of the chromosomes have divided, and a first-division anaphase in which 13 chromosomes are approaching each pole.

For *Sidalcea neomexicana* A. Gray, of the tribe Malveae, Tjebbes (12) found the haploid chromosome number 13, the basic chromosome

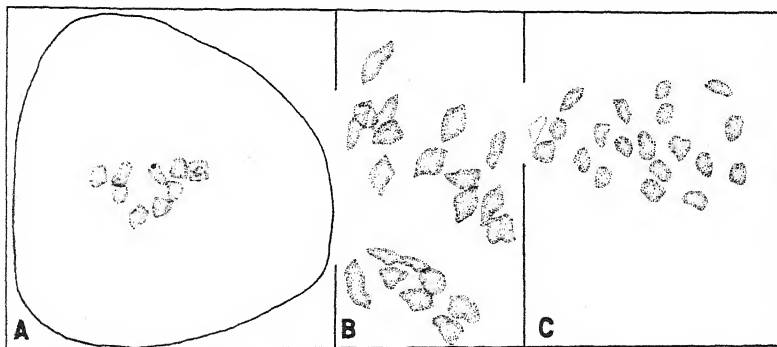


FIGURE 5.—Chromosomes from pollen mother cells of *Gossypium* relatives ( $\times 1,500$ ): A, Early first-division metaphase of *Cienfugosia heterophylla*; B, early first-division metaphase of *Hibiscus militaris*; C, early first-division metaphase of *H. palustris*

number of the genera *Gossypium*, *Thurberia*, and *Shantzia*, of the tribe Hibisceae.

Figure 5, A to C, shows the chromosomes in three other representatives of the tribe Hibisceae. *Cienfugosia heterophylla* (Vent.) Garcke, a species native to the southern part of the United States, has 10 bivalent chromosomes as shown in a first-division metaphase. (Fig. 5, A.)

Figure 5, B and C, shows the chromosomes from pollen mother cells of two representative *Hibiscus* species, *H. militaris* Cav. and *H. palustris* L. Counts were unusually difficult to make. Although 19 seemed to be the haploid chromosome number for both species, each figure shows a shadowy outline of what may be a twentieth chromosome. Youngman (13) studied the chromosomes of the species *H. rosa-sinensis* L., *H. tricuspes* Banks, and *H. tiliaceus* L. He experienced considerable difficulty in making counts, but from his best preparations he gives the diploid chromosome numbers 72, 40, and 48 for the three species, respectively.

For *Laratera thuringiaca* L. and *Malva moschata* L., of the tribe Malveae, Svensson-Stenar (11) found the haploid chromosome num-

ber 20. He gives the haploid chromosome number of *Malva palmata* Hedl. as approximately 20 and that of *M. pusilla* Sm. as between 20 and 30. These numbers are in harmony with the numbers found for *Cienfugosia* and *Hibiscus* of the tribe Hibisceae. The chromosome number 21 for *Malvastrum capense* Garcke, however, must be an aneuploid representative if it belongs to any of the chromosome series already described for Malvaceae.

#### POLLEN STERILITY

In conjunction with chromosome studies and variations in the reduction phases of the pollen mother cell, the pollen tetrad often is of considerable interest. Normally there are four equal-sized daughter cells in the tetrad. A deviation in the size or number of daughter cells represents an abnormality. If there is a variation in the size of the cells it is generally attributed to an unequal distribution of the chromosomes in either the first or second division. The presence of more than four cells shows not only that there has been an unequal distribution of the chromosomes but also that some chromosomes have failed to be included in the 2-daughter nuclei of the first division or in the 4-daughter nuclei of the second division. These extruded chromosomes develop into micronuclei and small pollen grains.

As a rule, cottons have only a small percentage of abnormal tetrads and the mature pollen looks uniform in size and shows only a very few sterile grains. In plants with a large percentage of sterile pollen a considerable number of tetrads were found to be abnormal. In a crinkly-leaf variant of the Pima variety (*Gossypium barbadense*?), for example, 15 per cent of the tetrads were abnormal and approximately 50 per cent of the pollen sterile. Some off-type plants, however, showed little sterile pollen. One plant, although showing considerable sterile pollen, had apparently normal tetrads. Abnormalities in the tetrads of a plant show that the chromosome behavior during pollen formation has been abnormal, and in such a plant there will be an appreciable amount of pollen sterility. The presence of sterile pollen in a plant, however, does not always indicate abnormal chromosome distribution and abnormal tetrads, for in the rogue mentioned above and in occasional plants in  $F_2$  progenies of *Acala*  $\times$  *Pima* hybrids, considerable sterile pollen was found that could not be traced to abnormalities in the chromosome distribution at the time of pollen formation.

#### SUMMARY AND CONCLUSIONS

Recent studies of the chromosome numbers in *Gossypium* made by the writer and by other investigators show the existence of two distinct groups, namely, the Asiatic group, in which the haploid chromosome number is 13, and the American group, in which the haploid chromosome number is 26. The separation of *Gossypium* species into two groups based on chromosome number corresponds with the general division of the species based on taxonomic characters.

This classification applies to the cultivated cottons and does not take into account certain wild species of *Gossypium*. Two of these, *Gossypium davidsoni* and *G. harknessi*, although indigenous to North America, have 13 chromosomes, the number characteristic of Old World cottons.

It is customary to consider the diploid representatives of a group of plants as being more nearly related to the ancestral type than those forms having higher chromosome numbers. Five species, so isolated that there has been little opportunity for contamination with their close relatives, may be considered as representing most nearly the ancestral type. These are *Gossypium stocksii*, *G. sturtii*, *G. davidsonii*, *G. harknessii*, and *Thurberia thespesioides*. The last, although usually considered as a separate genus, is very nearly related to *Gossypium*. All these species grow in more or less isolated localities and hence may represent the primitive stocks from which the cultivated varieties of cotton have arisen. The fact that they have only 13 chromosomes indicates that 13 was the haploid and 26 the diploid chromosome number of the ancestors of our present-day cottons.

The double chromosome number found in the American cottons suggests a duplication of the chromosomes of an ancestral type. Cytologically this has not been demonstrated. If *Gossypium* species having 26 chromosomes are true tetraploids, simple Mendelian ratios should appear much less frequently in them than in the Asiatic group. Matsuura (9) has brought together the studies on inheritance in *Gossypium*. These show that about half of the characters studied in the American group segregate in a 3:1 ratio, while in the Asiatic group all but two characters studied are distributed in a 3:1 ratio. Such a method of comparison is not conclusive evidence of the duplication of chromosomes in all species with 26 chromosomes, but it at least suggests that the American species of *Gossypium* are tetraploid.

Many of our American cottons have had ample opportunity to cross with each other. It is possible that their high chromosome number has arisen through hybridization and that this new stock of vigorous hybrids has obliterated many of the ancestral types. If American cottons are both polyploid and crypt hybrids, it is to be expected that they will show the same difficulties of taxonomic classification encountered in many polymorphic genera, in which intermediate forms often bridge the gap between distinguishing specific characters.

Of the other genera of the Malvaceae investigated, only *Thurberia* and *Shantzia* were found to have chromosome numbers suggesting a close relationship to *Gossypium*. The numbers in other Malvaceae are, however, distinctive, and a critical study of representatives of other genera may lead to the discovery of chromosome numbers indicating relationships.

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# THE VITAMIN A CONTENT OF ALFALFA AS AFFECTED BY EXPOSURE TO SUNSHINE IN THE CURING PROCESS<sup>1</sup>

By MARGARET CAMMACK SMITH, *Nutrition Chemist*, and IAN A. BRIGGS, *Assistant Agronomist, Arizona Agricultural Experiment Station*

## INTRODUCTION

Experiments in this laboratory have shown the value of alfalfa as a source of vitamin A in animal rations composed largely of the sorghum grain Hegari (6)<sup>2</sup>. In 1925 Steenbock and his coworkers (10) reported a loss in vitamin A accompanying the bleaching of clover hay due to excessive exposure to sunlight. The association of vitamin A with carotinoid pigment was pointed out at that time and since then considerable evidence showing the similarity in distribution of vitamin A and yellow or green coloring material has been accumulated (7, 8, 9, 11). More recently Moore (1, 2) has proved rather conclusively that carotene in plants is converted into vitamin A in the animal body and perhaps can be considered the precursor of vitamin A as ergosterol is the precursor of vitamin D. The inferior vitamin A value of discolored, bleached alfalfa is therefore to be expected.

It was the purpose of the work described in this paper to measure the comparative destructive effect of curing periods of different length on the vitamin A content of alfalfa produced in Arizona. Since this work was begun, Russell (3) has reported that machine-dried alfalfa contained about seven times as much vitamin A as that cured on the field in the usual manner in New Jersey.

## EXPERIMENTAL PROCEDURE

### PREPARATION OF THE ALFALFA

The alfalfa used was produced on the Salt River Valley farm at Mesa, Ariz. It was of the Hairy Peruvian variety, and of the third cutting from a planting in its second crop year. The alfalfa was growing thriftily, the plants were about 22 inches high, and the stand was thick. The soil on which the crop was produced is classified as Maricopa silt loam and definitely is alkaline in reaction.

The hay was cut with a mower at 11.15 a. m., June 24, 1929, that used in the experiment all being cut within the space of a few minutes. Immediately after the hay was cut, portions were spread out on the surface of the swaths so that the individual stalks were separated and did not overlap. This was done to secure maximum exposure to the sun. The spreading was done by four men and approximately one hour was required for the work. Care was taken to use only the stalks from the portions of swath most exposed to the sunlight after the first few minutes.

One sample was taken to a darkened curing house immediately after it was cut. All samples were placed on racks in this curing shed as soon as removed from the field and curing was completed in the shade in the absence of either sunlight or daylight. Windows

<sup>1</sup> Received for publication May 17, 1932; issued March, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 233.

were darkened so that very little light reached the curing hay. Ventilation was provided so that the hay dried without spoilage or discoloration.

The samples of alfalfa were removed from the field as shown in Table 1.

TABLE 1.—*Time of removing alfalfa, cut at 11.15 a. m., June 24, 1929, and period during which it remained in the field*

Sample No.	Date and hour removed from the field	Hours in the field	Sample No.	Date and hour removed from the field	Hours in the field
1.....	June 24, 11.15 a. m.....	0	4.....	June 24, 6 p. m.....	6 $\frac{3}{4}$
2.....	June 24, 2 p. m.....	2 $\frac{3}{4}$	5.....	June 25, 8 a. m.....	20 $\frac{3}{4}$
3.....	June 24, 4 p. m.....	4 $\frac{3}{4}$	6.....	June 25, 12 m.....	24 $\frac{3}{4}$

The day on which the hay was cut and the following day were classed as "clear" in the weather records. There was a slight haze the first of the two days which may have interfered with the intensity of the rays of the sun to some extent. The temperature was higher on the day the alfalfa was cut than on any other day of that year. A temperature of 115° F. was recorded at noon of that day. The meteorological data for the two days are shown in Table 2.

TABLE 2.—*Meteorological data for days on which alfalfa samples were cut*

Day	Air temperature		Wind movement	Evaporation
	Maximum	Minimum		
	° F.	° F.	Miles per hour	Inch
June 24.....	115	66	20	0.444
June 25.....	110	71	21	.372

Only the leaves of the alfalfa were used in the laboratory experimental work so as to insure more uniform samples. The leaves were carefully separated from the stems and petioles by hand, finely ground, and kept in covered glass jars in the laboratory until used.

#### METHOD OF MEASUREMENT OF VITAMIN A CONTENT

The method of measuring the vitamin A content of the different samples of alfalfa allowed to lie on the field for different periods of time is that developed by Sherman and Munsell (5) and modified somewhat by Sherman and Burtis (4). Albino rats taken from Sherman's diet B at the time of weaning were placed on a vitamin-A-free ration composed of 67 per cent cornstarch, 10 per cent dried yeast, 18 per cent alcohol-extracted casein, 4 per cent Osborne and Mendel's salt mixture, and 1 per cent sodium chloride. Vitamin D was provided by the incorporation of 0.5 g<sup>3</sup> of irradiated cholesterol in each 1,000 g of diet. When the store of vitamin A present in the bodies of the rats at weaning was exhausted, they were placed in individual round metal cages with screen bottoms and given the basal diet and distilled water ad libitum. Weighed amounts of the

<sup>3</sup>g is the abbreviation for gram or grams.

ground alfalfa leaves to be tested were fed daily for an experimental period of eight weeks. Record was kept of the weekly weights and general condition of each animal. At least two representative animals from each litter were kept as negative controls. At the end of the test period the animals still surviving were chloroformed, autopsy observations of such evidences of vitamin A deficiency as infection in glands at the base of the tongue, eyes, ears, sinuses, bladder, etc., were noted. These findings, in conjunction with the growth response to the alfalfa supplements, were used as criteria of the comparative amounts of vitamin A in the different alfalfa samples fed.

#### EXPERIMENTAL DATA AND DISCUSSION OF RESULTS

The summarized results of measurement of the vitamin A values of samples of alfalfa which have been cured on the field for different lengths of time appear in Table 3.

TABLE 3.—*Summarized results of feeding alfalfa leaves, differently treated in the field, to albino rats as the sole source of vitamin A*

Sample No.	Field treatment of alfalfa	Rats used	Average weight at end of fore-period	Alfalfa fed daily	Average gain (+) or loss (—) of rats in 8-week test period	Health and autopsy observations
		Number	Grams	Grams	Grams	
1	Removed as soon as cut and cured in the dark.....	6	125.0	0.005	+22.3	Slight infection in 2 cases.
		8	122.0	.0075	+41.8	No symptoms of vitamin A deficiency.
		10	134.7	.01	+61.7	Do.
2	Spread for 2¾ hours.....	2	122.6	.005	(a)	Severe infections in all cases.
		8	125.0	.0075	+19.0	Some infection in 3 cases.
		8	130.2	.01	+36.3	No symptoms of vitamin A deficiency.
		2	118.0	.005	—1.8	Severe infections in all cases.
3	Spread for 4¾ hours.....	8	123.6	.0075	+17.0	Some infection in 4 cases.
		8	127.0	.01	+39.5	No symptoms of vitamin A deficiency.
		2	119.6	.005	+9.0	Severe infections in both cases.
4	Spread for 6¾ hours.....	8	122.5	.0075	+24.1	Slight infections in 3 cases.
		8	117.3	.01	+35.0	No symptoms of vitamin A deficiency.
		4	129.0	.0075	(a)	Severe infections in all cases.
5	Spread for 20¾ hours.....	8	122.0	.01	+2.8	Severe infections in 6 cases.
		8	119.0	.02	+18.0	Severe infections in 4 cases.
		6	125.6	.03	+39.5	No symptoms of vitamin A deficiency.
		3	124.0	.0075	(a)	Severe infections in all cases.
6	Spread for 24¾ hours.....	8	126.2	.01	(a)	Do.
		6	126.5	.02	—9.2	Do.
		6	119.3	.03	+17.0	Some infections in all cases.
		2	131.2	.05	+49.0	No symptoms of vitamin A deficiency.
7	On field in swath for 1 week.....	2	100.0	1.02	(a)	Severe infections in all cases.
		4	107.0	.05	(a)	Do.
		6	106.0	.1	—8.0	Do.
		6	112.0	.2	+36.2	No symptoms of vitamin A deficiency.
8	Negative controls.....	25	122.6	0	(a)	Severe infections in all cases.

<sup>a</sup> Died before end of test period.

Alfalfa which was removed from the field as soon as cut and dried in a darkened house is seen to be a very excellent source of vitamin A. Five milligrams of these alfalfa leaves fed daily were sufficient to induce only slightly less gain than 3 g a week during the 8-week test period, which is Sherman's vitamin A unit gain. A gain of 41.8 g and 61.7 g in the test period, or of approximately 5 and 8 g per week, resulted from the daily feeding of 7.5 and 10 mg, respectively, of the alfalfa leaves prepared in the same fashion.

When, however, the alfalfa was spread out in the field for periods ranging in length from 2¼ to 6¼ hours 5 mg of the leaves failed to provide enough vitamin A to permit even the low rate of gain of 3 g a week. The animals dependent upon these samples of alfalfa fed at this low level for their vitamin A were not protected against the severe infections characteristic of vitamin A deficiency. All of the animals in this group showed signs of ophthalmia varying in degree from + to ++++ before death, and autopsies revealed infection in glands at the base of the tongue, ears, and sinuses. Increasing the quantity of alfalfa fed to 7.5 mg daily provided sufficient vitamin A to induce slightly less than the unit rate of gain (17–24 g in eight weeks). Only slight infections of eyes and sinuses were observed in these groups of animals. Again when the quantity of these three samples of alfalfa fed daily was further increased to 10 mg, not only was an average gain of from 35 to 39.5 g promoted in the three groups of animals, but all of the animals were free from the infections usually accompanying a marked vitamin A deficiency.

None of these samples of alfalfa had suffered loss of green color indicative of bleaching, and observable to the naked eye, as a result of their exposures on the field. In spite of this fact it is obvious that some destruction of vitamin A did result during this period, because it required the daily feeding of from one-fourth to one-half as much again to induce the same rate of gain in the test animals as was obtained by the feeding of the alfalfa cured in the dark. These alfalfa leaves which were exposed to the sun over the noon hour, 2¼ hours, therefore, contained from 20 to 33 per cent less vitamin A than those which had been removed from the field immediately after cutting and cured in the dark. However, no significant difference in vitamin A content resulting from 2¼, 4¼, and 6¼ hours of exposure was detected in these experiments.

When the alfalfa was allowed to lie on the field overnight, 11.15 a. m. one day until 8 a. m. the next, or a period of 20¼ hours, a decidedly greater degree of vitamin A destruction, again basing the comparison on the vitamin A content of the cured in the dark sample, was observed. In this case, even 10 mg daily failed to induce gains or protect the test animals from infections attributable to vitamin A deficiency. Twenty milligrams of the material was necessary approximately to produce the vitamin A unit gain in weight, and 30 mg were necessary to induce a growth of 39.5 g in the experimental period. These results are indicative of approximately 75 per cent destruction as a result of this period of exposure.

With an additional four hours of exposure to morning sunshine on the second day, the destruction was even greater. Roughly only 16 per cent of the vitamin A present in the sample cured in the dark was retained in the alfalfa dried in the dark after being spread out in the field for 24¼ hours. That both the 20¼-hour and the 24¼-hour samples had suffered some bleaching was readily observed, especially in the stems.

The rate of destruction is more rapid at first and then decreases as the time of exposure is extended. Alfalfa exposed in the swath for one week, during which time it received 57 hours of sunshine and 0.37 inch of rain, was severely bleached and retained only approximately 4 per cent of the vitamin A found in the sample cured in the dark.

These experiments supply further evidence that the conditions of the curing process bear a marked relation to the vitamin A content of alfalfa hay. In order to retain maximum vitamin A potency and thus best to preserve its nutritional value, it is evident that exposure to Arizona sunshine should be reduced to a minimum, and the alfalfa cured as quickly as possible to inhibit destructive oxidative or enzymatic changes.

#### SUMMARY

The vitamin A value of alfalfa leaves which have been allowed to lie spread out in the field for varying lengths of time has been compared with the vitamin A content of alfalfa leaves taken from the field immediately upon cutting and dried in a well-ventilated darkened house.

A loss of from 20 to 33 per cent of the vitamin A found in the sample cured in the dark resulted from allowing the alfalfa to lie carefully spread out on the field for the 2¾-hour period from 11.15 a. m. to 2 p. m. No greater degree of destruction was observed when the time of exposure was increased to 6¾ hours in the same day. No loss of green color in these alfalfa leaves could be observed.

A 75 per cent loss of vitamin A occurred when the alfalfa was allowed to lie on the field under the same conditions overnight, and until 8 a. m. the next day. This loss was increased to approximately 84 per cent upon a further 4-hour period of exposure until 12 noon the second day. These alfalfa leaves retained a large part of their green color, although the stems showed evidence of marked bleaching, but only 16 per cent of the vitamin A present in the sample cured in the dark was retained.

Alfalfa severely bleached as a result of 1-week exposure to sun and rain on the field in the swath retained but 4 per cent of the vitamin A present in the sample cured in the dark.

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# THE ANTIRACHITIC VALUE OF ALFALFA AS AFFECTED BY EXPOSURE TO SUNSHINE IN THE CURING PROCESS<sup>1</sup>

By MARGARET CAMMACK SMITH, *Nutrition Chemist*, and IAN A. BRIGGS, *Assistant Agronomist, Arizona Agricultural Experiment Station*

## INTRODUCTION

Alfalfa, a roughage widely used in animal rations, is now valued for its vitamin A content and calcifying properties.

The antirachitic properties of hays were first demonstrated to be related to their exposure to sunshine by Steenbock et al. in 1925 (7).<sup>2</sup> They showed that clover hay when made in the sunlight possessed calcifying properties which hay cured in the dark did not have. As Arizona receives a high percentage of the total possible sunshine all the year round, it seemed desirable to investigate the vitamin D content of Arizona-grown alfalfa, and to study the relation between curing periods of different lengths, under field conditions, and the calcifying properties of the hay.

Since this work was begun, Russell (4) of the New Jersey Agricultural Experiment Station has reported that artificially cured alfalfa leaves contained only small quantities of the antirachitic vitamin, but when alfalfa was dried in the sun without exposure to dew or rain there was an increase in the antirachitic potency of the leaves. This is in agreement with the findings of Steenbock and his coworkers.

## EXPERIMENTAL PROCEDURE

### MATERIALS USED

The alfalfa used in this study was prepared as described in detail in a previous paper (5). It was originally planned to measure the antirachitic value of all the samples used in the vitamin A study, but the early results obtained did not justify such an intensive study from the practical standpoint. Accordingly results reported in this paper deal with only three samples of alfalfa.

Sample No. 1 was taken from the field as soon as cut, 11.15 a. m., July 8, 1930, and cured in a dark well-ventilated room.

Sample No. 2 was spread out by hand and allowed to lie on the field from 11.15 a. m. one day (July 8) to 12 noon the next day, during which time it received 15 hours and 15 minutes of exposure to sunshine of varying intensity.

Sample No. 3 was allowed to lie on the field in the swath for one week. The maximum temperature during this period ranged from 93° to 104° F., the minimum temperature from 67° to 79°. During this 1-week period the sun shone 57.3 hours, but the alfalfa received only 42 per cent of the possible sunshine, for several of the days were cloudy and there was 0.37 inch of rainfall.

<sup>1</sup> Received for publication May 17, 1932; issued March, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 240.

In order to insure uniformity of sampling only the leaves of the alfalfa were used, as was the case in the previous experiments. The leaves were carefully separated from the stems by hand and finely ground.

#### MEASUREMENT OF THE ANTIRACHITIC POTENCY

The rickets curative method, essentially that suggested by the work of McCollum (3) and his coworkers, was used to measure the comparative antirachitic value of alfalfa samples cured for different lengths of time. Albino rats weighing from 55 to 64 g<sup>3</sup> were taken from the stock colony at the time of weaning and were placed upon Steenbock's (6) high calcium-low phosphorus rachitic ration No. 6529, composed of 76 per cent ground yellow corn, 20 per cent wheat gluten, 3 per cent calcium carbonate, and 1 per cent sodium chloride.

The rats, when kept in the dark, regularly become rachitic on this ration in 21 days, as evidenced by their enlarged joints and rachitic gaits. At this time a Shipley (3) "line test" on the proximal end of the tibia of a representative animal of each litter showed a wide metaphysis typical of severe rickets.

The remaining rats in each litter were then placed in individual metal cages with raised screen bottoms and given distilled water *ad libitum*. As some difficulty was encountered in getting the rats to eat quantitatively the ground alfalfa when fed separately, it was incorporated in the ration at a level of 5 and 25 per cent, replacing equivalent amounts of yellow corn. That the high calcium-low phosphorus ratio was not seriously disturbed was shown by analyses of the resulting rations showing a Ca and P ratio varying from 3.7 to 1 in diet 2965 to 4.7 to 1 in an experimental ration containing 25 per cent ground dried alfalfa leaves.

A record was kept of the food intake and daily gains in weight of each rat during the experimental test periods, and those animals refusing food and not gaining in weight were discarded. Several representative rats from each litter were kept upon the unsupplemented rickets-producing ration No. 2965 as negative controls, one control always being retained until the last rat in each litter had been chloroformed, its tibia dissected free from flesh and tested for rachitic lesions by means of the line test. If evidence of the so-called spontaneous healing of the rachitic lesions of the control animals was obtained, its litter mates were discarded. In the early fall some difficulty was experienced in the production of rickets in the usual 21-day period and at the same time, unexplainable cures of rickets in the control animals on the basal rachitic rations occurred. The recent findings of Harris and Bunker (2) that freshly ground corn has antirachitic properties which are destroyed during the aging process satisfactorily explain the difficulties encountered, for at the time a supply of the new crop of yellow corn was being used in the ration.

Degree of healing of the rachitic lesions was graded one to four positive (+ to +++) according to the method of Bills et al. (1).

Table 1 shows the results of feeding to rachitic animals dried ground alfalfa leaves taken from the field as soon as cut and cured in the dark.

<sup>3</sup>g is the abbreviation for gram or grams.

TABLE 1.—*Summarized results of feeding various quantities of alfalfa leaves, cured in the dark, to rachitic rats*

[The line-test findings denoted no healing in any case]

Percentage of alfalfa in the ration	Rats used	Length of test period	Average gain in weight in test period	Average daily food intake
	Number	Days	Grams	Grams
5.....	2	5	4.0	7.0
	7	7	7.0	6.4
	6	14	12.5	6.4
	2	21	18.0	6.4
15.....	1	28	18.0	8.5
	2	7	5.0	7.2
	2	7	5.0	6.8
25.....	6	5	4.3	8.2
	3	14	15.5	8.0
	6	21	21.7	9.0

Obviously the alfalfa leaves that were taken from the field immediately upon cutting and cured in the dark contained no appreciable amounts of the antirachitic vitamin, if any at all. Inclusion in the basal ration of as much as 25 per cent ground alfalfa leaves did not heal the rachitic lesions of the test animals even when fed over a period of three weeks. In no case did a line at the zone of provisional calcification appear when these alfalfa leaves were included in the ration.

As a further test of the absence of vitamin D in alfalfa which had not been exposed to sunlight, 18 rats representing three litters were selected at the time of weaning, half of them placed upon Steenbock's rachitic ration No. 2965, and the other half, under similar conditions, on the same ration in which 25 per cent ground dried cured in dark alfalfa leaves had replaced an equivalent weight of the yellow corn. At the end of 21 days the animals were killed and a line test was made upon the left tibia of each animal. Rickets of equal degree of severity was observed in all of the animals both on diet 2965 and the alfalfa ration, thus showing that 25 per cent alfalfa leaves in the ration neither prevented the development of rickets nor cured rachitic lesions that had already developed. In this manner further evidence of the low antirachitic potency of the alfalfa, when unexposed to sunlight, was obtained.

TABLE 2.—*Summarized results of feeding various quantities of alfalfa leaves, cured for 24 $\frac{3}{4}$  hours on the field, to rachitic rats*

Percentage of alfalfa in the ration	Rats used	Length of test period	Average gain in weight	Average daily food intake	Line-test findings
	Number	Days	Grams	Grams	
5.....	5	5	4.5	5.0	No healing in any case.
	4	7	8.0	6.0	Do.
	7	14	13.8	7.8	No healing in 5 cases, + healing in 2 cases.
	7	21	17.0	8.2	No healing in 5 cases, ++ healing in 2 cases.
	4	28	32.7	9.5	No healing in any case.
25.....	10	5	4.0	6.2	No healing in 8 cases, ++ healing in 2 cases.
	7	7	2.0	6.7	No healing in 6 cases, ++ healing in 1 case.
	2	10	9.0	-----	++ healing in both cases.
	12	14	11.7	8.3	++ healing in 2 cases, +++ healing in 5 cases, ++++ healing in 5 cases.

Following the same procedure, measurement was made of the antirachitic value of alfalfa sample No. 2 which had remained on the field from 11.15 a. m. one day until 12 noon the next day, a period of 24¾ hours, before being gathered.

The results are presented in Table 2.

From Table 2 it is clear that alfalfa which has been cured on the field for 24¾ hours in Arizona possesses calcifying properties that alfalfa cured without exposure to sun does not have. Even under these conditions the synthesis of the antirachitic vitamin has not been great, for the inclusion of 5 per cent of this alfalfa in the ration resulted in no healing of the rachitic lesions of rats in the majority of cases tested, and in but slight healing in 4 out of 27 cases. Again, it was found possible to produce rickets in rats that had been fed from the time of weaning upon the rickets-producing ration in which 5 per cent of the 24¾-hour-exposure alfalfa leaves were substituted for an equal weight of yellow corn. When these animals were kept in the dark, rickets of a degree only slightly less severe than that of the control animals on diet 2965 developed in the 21-day test period. That the calcifying properties of the alfalfa had been increased, however, by allowing it to lie on the field for 24¾ hours, was made evident by the inclusion of 25 per cent of the leaves in the basal ration. At this higher level of feeding healing was practically complete in a test period of two weeks, although it was not regularly obtained in the 5-day test period advocated by Bills (1).

Results of feeding to rachitic rats alfalfa sample No. 3 which had been allowed to lie in the swath for a week appear in Table 3.

TABLE 3.—*Summarized results of feeding various quantities of alfalfa leaves, cured for one week on the field, to rachitic rats*

Percentage of alfalfa in the ration	Rats used	Length of test period	Average gain in weight	Average daily food intake	Line-test findings
	Number	Days	Grams	Grams	
5	4	3	1.0	8.0	++ healing in all cases.
	7	5	4.0	7.4	++++ healing in 2 cases, +++++ healing in 5 cases.
	7	7	5.8	7.0	++++ healing in all cases.
	2	2	1.5		+ healing in 1 case, no healing in 1 case.
25	3	3	4.0	8.0	+++ healing in 2 cases, +++++ healing in 1 case.
	7	5	6.0	6.2	++++ healing in 6 cases, +++ healing in 1 case.
	11	7	6.5	6.4	++++ healing in all cases.

The longer period of exposure in the curing process of the alfalfa resulted in marked increase in its calcifying properties. Sufficient vitamin D had been synthesized to induce complete calcification of the rachitic bones, in the majority of the rats tested, in a period of five days when the alfalfa leaves fed were at the low level of 5 per cent.

## DISCUSSION

Further evidence that the antirachitic potency of alfalfa hay is directly related to the duration of its exposure to sunlight is presented by the data tabulated above. The conditions of the curing

process are therefore of considerable importance, for increasing the calcifying properties of alfalfa enhances its feeding value.

Samples of alfalfa which had been cured for shorter periods than 24 hours were not tested for vitamin D as at first planned for synthesis of vitamin D in the 24-hour curing period was not of such a high degree to make it seem worth while to test alfalfa which had even less opportunity for this synthesis.

In spite of the fact that allowing alfalfa to lie on the field after cutting undoubtedly increases its calcifying properties in proportion to the length of exposure to sunshine, this does not seem a justifiable procedure from the practical standpoint. In a previous paper (5) it has been shown that destruction of vitamin A increases very greatly as the time of exposure is prolonged. The alfalfa allowed to lie on the field under the conditions of this experiment showed some increase in its antirachitic potency, but there was an accompanying destruction of practically 84 per cent of the vitamin A content, although bleaching of the green color was not excessive. When synthesis of vitamin D was great, as in the first week of exposure, the alfalfa was severely bleached and comparatively devoid of vitamin A.

The curing of alfalfa in Arizona can perhaps be considered more of a dehydration than a maturing process. It is the usual custom of the farmers in this State to collect the alfalfa in windrows after it has wilted (several hours), and bale it from two to five days after cutting. The alfalfa as marketed, therefore, has in most cases been exposed to the direct sun's rays less than the alfalfa under test which was carefully spread out by hand and allowed to lie on the field from 11.15 a. m. one day until 12 noon the next day.

Increased exposure of alfalfa to sunlight for the purpose of enhancing its calcifying properties apparently is not a procedure to be recommended because of the accompanying marked destruction of vitamin A, as well as possible weather damage, loss of leaves, and other disadvantages.

### CONCLUSIONS

The data presented in this paper give additional evidence that alfalfa cured in the dark is deficient in antirachitic potency and that synthesis of vitamin D occurs when the alfalfa is exposed to sunlight in the curing process.

In the experiments described, prolonged feeding of the alfalfa cured in the dark resulted in no alleviation of the induced rachitic condition of the experimental rats.

Alfalfa that had been carefully spread out on the field from 11.15 a. m., one day to 12 noon the next, during which time the sun shone for 15 hours and 5 minutes, possessed mild calcifying powers.

Alfalfa which had been allowed to lie in the swath for one week, during which time the sun shone 57.3 hours and 0.37 inch of rain fell, was found to be highly antirachitic.

The practicability of increasing the vitamin D content of alfalfa and hence its feeding value by prolongations of its exposure to sunlight in Arizona is discussed, but such procedure is not recommended because of serious detriment to the hay.

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# INFECTION OF CORN PLANTS BY *PHYSODERMA ZEAEMAYDIS* SHAW<sup>1</sup>

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## INTRODUCTION

Brown spot has been reported as causing losses estimated at 5 to 10 per cent of the corn crop in certain sections in the South Atlantic and Gulf Coast States;<sup>3</sup> in the vicinity of Gainesville, Fla., the damage is estimated at 5 per cent. This disease, which is caused by the fungus, *Physoderma zeaemaydis* Shaw,<sup>4</sup> attacks the leaves, leaf sheaths, stalks, and occasionally the outer husks of ears of the corn plant. Tisdale<sup>5</sup> has given most of the information available on the nature, distribution, and economic importance of brown spot and the life cycle of the causal organism.

Since brown spot is one of the most important diseases of corn in the South, experiments were begun at Gainesville, Fla., in 1929 to obtain further information on the disease and to establish methods of procedure by which the relative susceptibility of varieties and pure lines of corn may be tested, preliminary to a study of the possibility of controlling this disease by developing resistant inbred lines which could be combined into synthetic varieties. This work has been in progress three years, and information has been obtained on (1) some of the outstanding characteristics of the disease, (2) an effective method of inoculating plants in the field and greenhouse to produce epidemic conditions, (3) longevity of the fungus and time required for the production of sporangia in host tissue, (4) influence of certain factors on the rate of infection, and (5) comparative susceptibility of certain commercial varieties and inbred lines to the disease.

## HOSTS

Thirty-eight southern varieties of *Zea mays* var. *indentata* Sturt. (dent corn) and *Z. mays* var. *indurata* Sturt. (flint corn) grown in test plots proved to be susceptible to brown spot, and infection experiments also showed that *Z. mays* var. *saccharata* Sturt. (sweet corn), *Z. mays* var. *evarta* Sturt. (pop corn), *Z. mays* var. *amylacea* Sturt. (flour corn), and *Z. mays* var. *tunicata* Sturt. (pod corn) are also susceptible to the disease. The fungus is a common parasite in the leaves of *Euchlaena mexicana* Shrad. (teosinte). Other plants that were artificially inoculated but failed to show brown-spot infection were: *Tripsacum dactyloides* L. (gama grass), and *Coix lachryma-jobi* L. (Jobs-tears) near relatives of the corn plant; and *Holcus sorghum* L. var. *saccharatus* (L.) L. H. Bailey (sorgo), *H. sorghum* L. var.

<sup>1</sup> Received for publication Apr. 20, 1932; issued March.

<sup>2</sup> Thanks are due F. H. Hull and J. D. Warner, assistant agronomists of the Florida Agricultural Experiment Station, for access to their experimental plots for the purpose of making observations.

<sup>3</sup> TISDALE, W. H. *PHYSODERMA DISEASE OF CORN*. Jour. Agr. Research 16: 137-154, illus. 1919.

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<sup>5</sup> TISDALE, W. H. Op. cit.

*technicus* (Körn. and Wern.) L. H. Bailey (broomcorn), *Pennisetum typhoides* Rich. (pearl millet), *Panicum miliaceum* L. (broomcorn millet), *Chaetochloa magna* (Griseb.) Scribn. (Everglade millet), *C. viridis* (L.) Scribn. (green foxtail), *C. lutescens* (Weigel) Stuntz (yellow foxtail) and *Saccharum officinarum* L. (sugarcane).

### SYMPTOMS

Since Tisdale <sup>6</sup> has described the manner of infection and symptoms of brown spot, only the outstanding characteristics of the disease are mentioned in this paper. Brown spot is most often located on the plant parts below the ear or even between the fourth node and the ground. In the most severe cases of infection, all or almost all the leaf blades and sheaths on a stalk become diseased and die prematurely, resulting in a weakened plant which bears only a small ear or none. (Fig. 1.) Leaf sheaths near the base of the plant are the most common points of infection, and the culm in this region is sometimes invaded, causing the plant to break, particularly if the disease has girdled it. (Fig. 2.) Leaf-blade infection occurs in four forms: (1) As alternating bands of diseased and healthy tissue (fig. 3, A); (2) as a chlorosis of the tips and in some cases all or a major portion of the entire blade, in which the infected areas are filled with small brown spots containing sporangia (fig. 3, B); (3) as brown areas at the junction of leaf blade and sheath sometimes extending up the midrib of the leaf and down into the sheath, which may become so severely diseased that the water and food supply is cut off, resulting in the wilting and premature death of the leaf (fig. 3, C); and (4) as small brown spots coalesced or scattered in the midrib of the leaf blade. All these forms of leaf infection may appear in one leaf or in different leaves of the same plant. The outer husks of ears are rarely infected, but the husks of rudimentary ears which have failed to emerge from the protecting leaf sheath are frequently diseased.

### INOCULATION EXPERIMENTS

Tisdale <sup>6</sup> obtained infection of corn plants by spraying a water suspension of sporangia of *Physoderma zeae-maydis* behind the leaf sheaths and into the buds, under certain environmental conditions. In testing the reaction of varieties and inbred lines of corn to brown spot, it is essential that they be exposed to the parasite under favorable conditions for infection to occur. Accordingly, inoculation experiments were conducted in the greenhouse in the winter of 1929-30 and in the field in 1930 to find a convenient and effective method of inoculating large numbers of plants.

#### IN THE GREENHOUSE

Corn plants were grown in 6-inch flowerpots in a greenhouse where the mean daily range in temperature was 68.5° to 96.6° F. and the mean daily range in relative humidity was 42.4 to 86.9 per cent. Equal numbers of plants 2 to 3 feet in height, of each of 10 selfed lines, which had been inbred from two to three generations, were inoculated

<sup>6</sup> TISDALE, W. H. Op. cit.



by each of two methods in which sporangia suspended in water were used as the inoculum. The results showed that 59.6 per cent of 248 plants inoculated by injecting the inoculum into the stalk at or near



FIGURE 1.—A Healthy plant bearing a normal-size ear; B, brown-spot-infected plant bearing no ear

the growing point became infected, while 32.2 per cent of a like number of plants became infected when the inoculum was poured into the upper whorl of leaves.

Sporangia suspended in water served as a better inoculum than dry sporangia. Of 40 plants inoculated with a sporangial suspension 23

developed brown spot, while only 7 of 40 plants developed the disease when dry sporangia were used. Apparently the dry sporangia failed to come in contact with the water in many plants, and thus failed to germinate and invade the host, whereas sporangia suspended in water not only had a more favorable environment for germination but were brought into closer contact with susceptible host tissues by the water of the inoculum moving downward in the crevices between the leaves of the plant.

Other experiments were conducted in which corn kernels and soil were inoculated by the following methods: (1) Dusting sporangia on the kernels; (2) plunging the kernels into a water suspension of sporangia; (3) mixing sporangia with the top 2 inches of soil; (4) dusting sporangia on the surface of the soil; and (5) filling the upper whorl of leaves of plants, 2 to 3 feet in height, with a water suspension of sporangia. Forty plants were used in testing each method of inoculation, and notes were taken on infection seven weeks after planting.

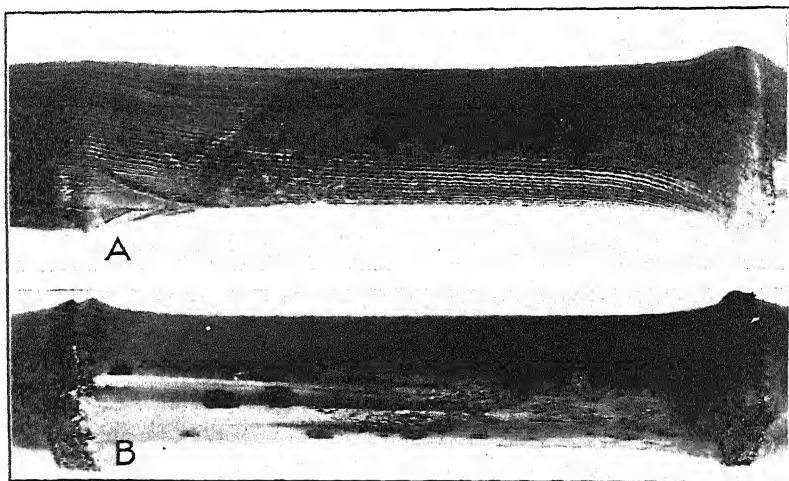


FIGURE 2.—Brown spot infection on sheath (A) and culm (B) of a corn plant

Twenty-three of the 40 plants inoculated by the fifth method developed brown spot, thus showing that the strain of corn used was susceptible to the disease and that the conditions maintained in the greenhouse were favorable for its development. No infection resulted when the other four methods of inoculation were used, although one plant growing in a pot in which the sporangia were dusted on the surface of the soil became infected. It is quite probable that infection in this case resulted either from sporangia that were splashed into the bud or sheath water of the plant when it was watered or from sporangia transported by air currents or insects from the surface of the inoculated soil to the plant.

It is reasonable to assume that under field conditions a much higher rate of infection would result if plants were grown in soil as heavily charged with sporangia as was the soil in which the plants were grown in the greenhouse. Soil in the field is stirred by frequent cultivation, and the top layer at times becomes very dry so that the wind can pick up fine soil particles to which sporangia adhere and deposit them on

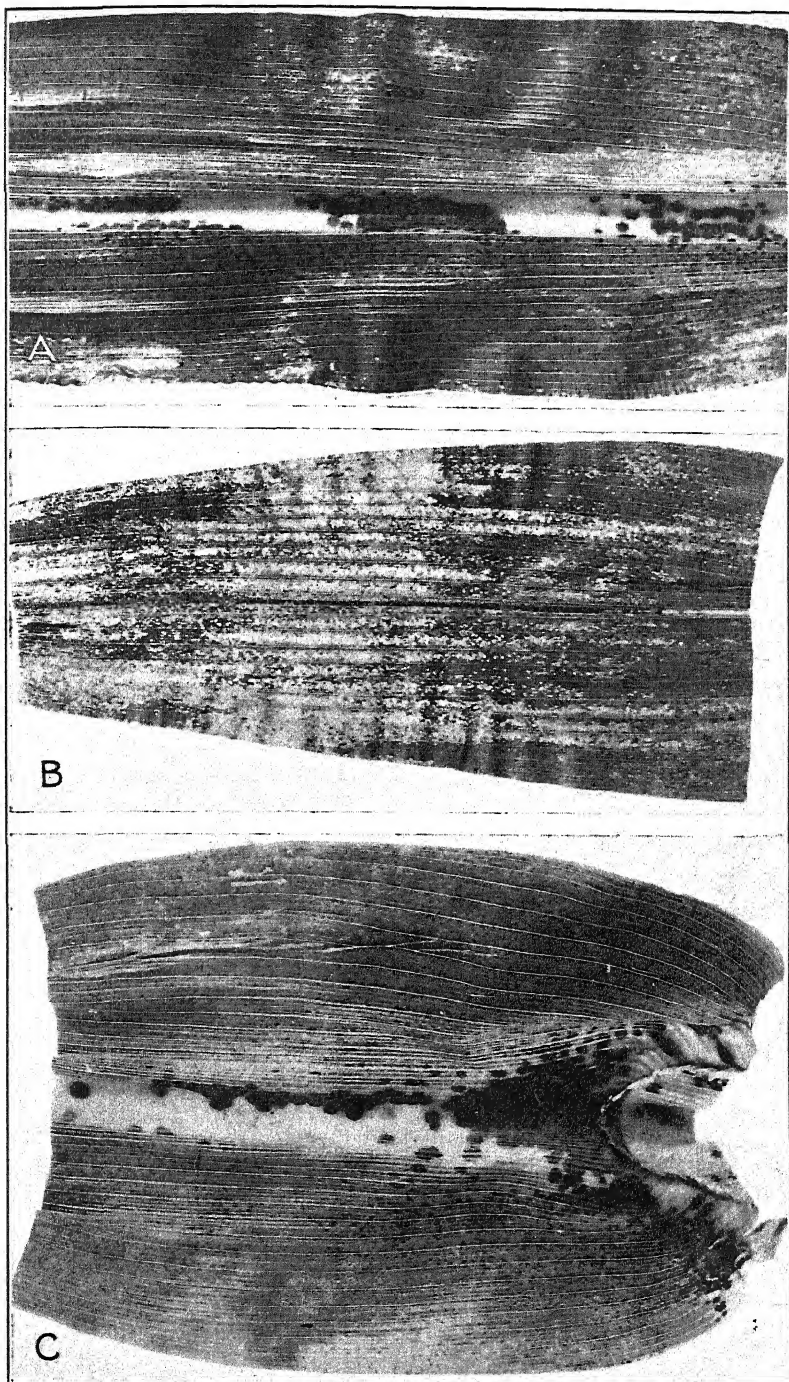


FIGURE 3.—Three forms of brown-spot infection of corn leaves: A, Leaf with alternating bands of diseased and healthy tissues; B, leaf with chlorotic areas near tip; C, leaf with brown areas near junction of blade and sheath

the plant. In the greenhouse the surface of the soil in the pots was not disturbed, and since it was watered daily, probably many of the sporangia were washed downward. The surface remained moist most of the time, and there was little movement of the air above the benches crowded with growing plants.

#### IN THE FIELD

Corn plants 2 to 3½ feet in height were inoculated with a water suspension of sporangia in the season of 1930 by three methods, as follows: (1) The upper whorl of leaves of each plant was filled to overflowing with inoculum from a quart-size pressure sprayer; (2) inoculum was sprayed on the stalks of the plants from the top to the bottom; and (3) 5 c c of inoculum was injected into the stalks of the plants at or near their growing points with a hypodermic syringe. At the end of 50 days the highest percentage of brown spot appeared in plants which had had the upper whorl of leaves filled with inoculum. (Table 1.)

The injection method gave the highest rate of infection in the greenhouse, and the probable explanation for its being less effective than other methods in the field is that the injections were not made at the right point in the stalks of the plants. In the greenhouse the plants were very uniform in size, and, since they were grown in pots, they could be handled easily, thus enabling the operator to locate fairly readily the growing points and make the injection. In the field the plants varied in size, and, since they could not be moved about, it was difficult to find the growing points and inject the inoculum into them.

TABLE 1.—Comparative effectiveness of three methods of inoculation in producing brown spot in corn plants in the field in 1930

Method of inoculation	Plants inoculated	Plants with brown spot at end of 50 days
	Number	Per cent
Sporangial suspension sprayed into the upper whorl of leaves of the plants.....	316	96.5
Sporangial suspension sprayed on the stalks of the plants.....	324	79.3
Sporangial suspension injected into the plants.....	327	74.8
Plants not inoculated (checks).....	315	12.7

#### COMPARATIVE EFFECTIVENESS OF GREENHOUSE AND FIELD INOCULATIONS

Comparisons were made of the percentages of brown spot developing in six inbred lines of corn artificially inoculated and grown in the greenhouse in the winter months of 1929-30 and in the field in the summer of 1930 to determine whether the inbred lines reacted the same toward the disease in the two places. The seed of each line planted in both places was from the same ear, and the plants that were in a susceptible stage of growth were inoculated by filling the upper whorl of leaves with a water suspension of sporangia of *Physoderma zeae-maydis*.

In these tests two lines, Wilson 57-39-1-3 and Boys Corn Club 65-1-5, were outstanding for susceptibility in the greenhouse, and,

although the percentage of infection was lower, they were also most susceptible in the field. The other four lines showed a comparatively low percentage of infection in the greenhouse and a much higher percentage in the field, although the order of their resistance was changed. Furthermore, the total percentage of plants infected in the field was almost twice that of those infected in the greenhouse, thus showing that the reaction of inbred lines to brown spot in the greenhouse is not a true indication of their reaction under field conditions. (Table 2.)

TABLE 2.—Comparison of the percentages of brown spot produced by artificial inoculation in inbred lines of corn grown in the greenhouse and in the field

Variety and inbred line number	Inoculated in green-house		Inoculated in field			
	Total plants	Infected plants		Total plants	Infected plants	
		Number	Per cent		Number	Per cent
Gist 2-3-13.....	36	6	16.6	77	55	71.4
Mathers 12-2-1.....	15	5	33.3	22	12	54.5
Cuban Yellow Flint×Florida Flint 18-2-8.....	47	12	25.5	89	41	46.1
Cuban Yellow Flint×Florida Flint 18-2-1-6.....	45	5	11.1	78	44	56.4
Wilson 57-39-1-3.....	31	27	87.1	63	52	82.5
Boys Corn Club 65-1-5.....	17	16	94.1	12	9	75.0
Total.....	191	71	37.2	341	213	62.5

### LONGEVITY OF SPORANGIA

Tisdale has shown <sup>8</sup> that sporangia of *Physoderma zeae-maydis* remain viable over winter in Alabama, Florida, Mississippi, and South Carolina. A test was started in 1928 to determine how long sporangia would remain capable of causing infection when kept under different environmental conditions. Sporangia were scraped from dead infected corn leaves and leaf sheaths, passed through sieves to remove fragments of host tissue, and then treated as follows: (1) Sporangia were mixed with potting soil, sandy loam soil, and sandy soil, and stored in test tubes which were plugged with cotton, sealed with paraffin, placed in an open flowerpot, and buried in the ground at a depth of 1 foot; (2) sporangia were stored in bottles kept on a shelf in a cabinet in the laboratory; and (3) sporangia in naturally infected leaf sheaths were placed in an open box nailed to the side of a building, exposed to the weather. Table 3 shows that sporangia stored in the three types of soil for three years produced brown spot, and that the sandy soil was a more favorable medium than was either the potting soil or the sandy loam soil. Sporangia kept in bottles in the laboratory for one and two years gave very high percentages of infection, but after three years there was a decrease in their ability to produce the disease. After two years in leaf sheaths the sporangia had lost little, if any, of their power to cause brown spot.

<sup>8</sup> TISDALE, W. H. Op. cit.

TABLE 3.—*Comparative percentages of infection obtained by inoculating corn plants in the field in 1931 with sporangia of *Physoderma zeae-maydis* of different ages and stored under different conditions*

Year sporangia were collected	Age of sporangia	Conditions of storage	Plants infected <sup>a</sup> by the sporangia
	Years		Per cent
1928.....	3	Potting soil.....	64
1928.....	3	Sandy loam soil.....	49
1928.....	3	Sandy soil.....	85
1930.....	1	Laboratory bottle.....	99
1929.....	2	do.....	94
1928.....	3	do.....	41
1929.....	2	Leaf sheath.....	87
Not collected (check).....	Unknown.	Soil and plant debris.....	4

<sup>a</sup> Each determination based on 100 inoculated plants of the Tisdale variety of corn.

By inoculating corn plants growing in the field in 1930 with sporangia taken from infected areas in green leaf sheaths, it was demonstrated that they will germinate and cause infection as soon as they are mature. Forty-one plants of the 90 inoculated with such sporangia developed brown spot, while only 6 of the 96 plants not inoculated became diseased.

In the main corn-growing section in north Florida it is believed that most of the brown spot developing during any one season is caused by sporangia produced one or more seasons previously, because sporangia are not liberated from the infected tissues of early-planted corn until the main crop is in an advanced stage of maturity and not susceptible to infection. It is possible that secondary infection takes place to some extent in the southern part of the State, as the planting season is longer there than in northern Florida. Sporangia probably can be carried great distances by the wind.<sup>9</sup> If so, some of the brown spot in corn fields in the northern part of the State may be due to sporangia produced in the southern part during the same year.

#### TIME REQUIRED FOR THE PRODUCTION OF SPORANGIA IN HOST TISSUE

Data were taken to determine the time required for sporangia to develop in inoculated plants grown in the greenhouse in the winter of 1929-30 and in the field in the summer of 1930. Inoculations were made in the greenhouse by the hypodermic-injection method and by filling the buds of the plants with a water suspension of sporangia. In the field only the latter method was used. When inoculated, plants in the greenhouse ranged in height from 1 to 3 feet and those in the field from 2 to 3½ feet.

As shown in Table 4, 11 days elapsed between the date of inoculation and the first appearance of sporangia in the infected tissues of corn plants growing in the greenhouse. The maximum number of plants with brown spots containing sporangia was observed on the twenty-fourth day. In the field, sporangial formation which could have been due to artificial inoculation was first observed on the sixteenth day after the date of inoculation, and there was a rapid increase in the rate of occurrence of sporangia from the seventeenth

<sup>9</sup> STAKMAN, E. C., HENRY, A. W., CURRAN, G. C., and CHRISTOPHER, W. N. SPORES IN THE UPPER AIR. Jour. Agr. Research 24: 599-606, illus. 1923.

to the twenty-fifth day. On the thirty-sixth day sporangia had been formed in diseased areas in 294 of the 315 inoculated plants, while only 7 of the 315 uninoculated plants were diseased.

In both the field and greenhouse the first sign of brown-spot infection was the development of areas which were a darker green than the normal healthy tissue. (Fig. 4.) Two to three days later the spots turned dark brown, indicating the presence of sporangia.

The variation in the time required for the disease to become established and for the formation of sporangia in the tissues of corn plants after inoculation is probably due to the variation in time

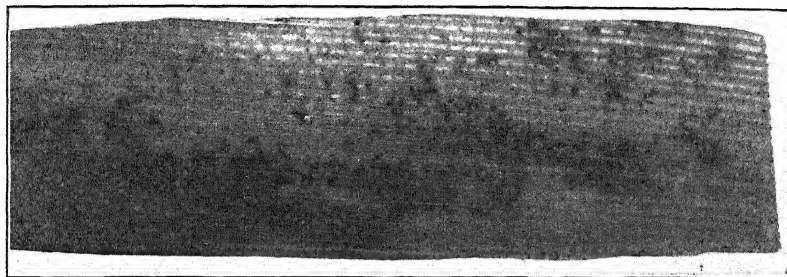


FIGURE 4.—An early stage in the development of brown spot in a corn-leaf sheath. The infected areas are a darker green than the uninfected tissue and the dark-brown spots containing sporangia are beginning to appear

required for the sporangia to become exposed to conditions favorable for germination and subsequent invasion of the host tissues.

TABLE 4.—Time required for the development of sporangia of *Physoderma zae-maydis* in artificially inoculated corn plants growing in a greenhouse and in the field

Days after inoc- ulation	Number of plants in which sporangia were present in—			Days after inoc- ulation	Number of plants in which sporangia were present in—		
	Green- house (165 inocu- lated)	Field			Green- house (165 inocu- lated)	Field	
		315 inocu- lated	315 unin- oculated			315 inocu- lated	315 unin- oculated
10	0	-----	-----	22	49	177	1
11	3	-----	-----	23	50	209	2
12	5	-----	-----	24	58	228	2
13	6	-----	-----	25	58	256	2
14	9	0	0	26	58	263	2
15	11	1	1	27	58	-----	-----
16	16	2	1	28	58	269	2
17	24	4	1	29	(a)	278	6
18	28	67	1	32	(a)	287	7
19	34	80	1	34	(a)	290	7
20	35	-----	-----	36	(a)	294	7
21	48	128	1				

<sup>a</sup> No observations made.

#### RELATION OF STAGE OF GROWTH OF PLANTS TO INFECTION

To determine whether there is a relationship between the age and height of corn plants and their susceptibility to brown spot in the field, plants were inoculated at different stages of growth during the seasons of 1929 and 1930. In 1929, 80 per cent of 217 plants with



their tassels emerging, developed brown spot when their stalks were sprayed with a suspension of sporangia, while only 28 per cent of a like number exposed to natural infection developed the disease. In 1930, five plantings of a commercial variety were made at weekly intervals from April 18 to May 16, and all the plants were inoculated with a water suspension of sporangia of *Physoderma zeae-maydis* on June 9. As noted in Table 5, the highest percentages of infection occurred in plants inoculated when they were 38 to 52 days old and measured 30 to 51 inches in height. Infection in plants 24 and 31 days old and 10 to 12 inches high was the same and was from 30.6 to 36.4 per cent less than that in older and taller plants.

The results of the inoculation experiments show that corn plants in the field are most susceptible to brown-spot infection when 45 to 52 days of age and that infection may occur at any stage of growth, beginning when the plants are 10 inches in height and including plants that have reached the tasseling stage. It is possible that infection may occur when plants are less than 10 inches in height, but it is not likely that it occurs after the plants have tasseled, for their tissues begin to mature and harden at this stage and this would prevent the fungus from entering.

In the greenhouse, plants 5 to 6 weeks old and 2½ to 3 feet in height were more susceptible to brown spot than were those inoculated when they were in an earlier or a later stage of growth. The disease failed to develop in plants inoculated when they were less than a foot high.

TABLE 5.—Comparative susceptibility of corn plants of different ages to *Physoderma zeae-maydis* in the field in 1930<sup>a</sup>

Date of planting	Age of plants	Average height	Inoculated		Not inoculated	
			Total infected		Total infected	
	Days	Inches	Number	Per cent	Number	Per cent
Apr. 18.....	52	51	121	97.5	107	33.6
Apr. 25.....	45	35	74	98.6	56	10.7
May 2.....	38	30	84	92.8	85	10.5
May 9.....	31	12	53	62.2	82	0
May 16.....	24	10	81	62.2	69	1.4

<sup>a</sup> All plants were inoculated June 9, 1930, and final notes on infection were taken July 9, 1930.

#### RELATION OF WEATHER CONDITIONS TO INFECTION

Ten to ninety per cent or more of the corn plants in fields in the vicinity of Gainesville may become infected with brown spot. In the main crop planted in March and early April farther north the disease usually makes its first appearance the latter part of May or the first of June, and maximum infection is usually reached on or before July 15.

According to Tisdale,<sup>10</sup> conditions most likely to cause an epidemic of brown spot in a cornfield where the plants are making a normal growth are (1) the presence of the sporangia of the causal organism, (2) daily temperatures ranging between 23° and 30° C., and (3) the retention of the sheath and bud water in the plants until the sporangia can germinate and cause infection. Weather conditions favoring the maximum development of the disease at Gainesville usually prevail during May and June.

<sup>10</sup> TISDALE, W. H. Op. Cit.



Comparisons were made of the percentages of brown spot in 10 varieties of corn planted March 10, 1930, and March 6, 1931, and grown in the same field on the agronomy farm in both seasons. Nineteen per cent more of the plants were infected with brown spot in 1930 than in 1931, the infection being 53 and 34 per cent, respectively, for these two years. The temperatures in April and May, 1930, were higher than for the same period in 1931; and there was much more rainfall, a greater number of showers, partly cloudy, and cloudy days during the period from April 1 to June 30, 1930, than there were during this same period in 1931 (Table 6), which verifies Tisdale's conclusion<sup>11</sup> that the disease is more prevalent in warm, wet seasons.

TABLE 6.—*Weather conditions prevailing in April, May, and June, 1930 and 1931, at Gainesville, Fla.*

Condition	April		May		June	
	1930	1931	1930	1931	1930	1931
Clear days.....number.....	9	15	8	14	5	16
Partly cloudy days.....do.....	15	10	22	16	14	13
Cloudy days.....do.....	6	5	1	1	11	1
Days precipitation was 0.01 inch or more.....number.....	6	7	6	10	20	9
Total precipitation.....inches.....	2.12	2.83	4.99	2.85	12.03	1.53
Mean maximum temperature.....° F.....	80.20	78.37	89.22	85.40	85.86	92.57
Mean minimum temperature.....do.....	57.70	55.00	63.55	61.08	68.30	67.50
Mean temperature.....do.....	68.90	66.70	76.38	73.26	77.08	80.03

#### COMPARATIVE SUSCEPTIBILITY OF VARIETIES OF CORN TO BROWN SPOT

None of the varieties of dent and flint corn tested have been found to be immune to brown spot. The disease has been observed in each of 38 varieties planted March 10, 1930, and March 6, 1931, in test plots at Gainesville, Fla. However, these varieties reacted differently to brown spot; in 10 varieties that were grown in both years, infection in 1930 varied from 33.3 to 78.2 per cent, and from 14 to 46 per cent in 1931, as shown in Table 7. The weather conditions in 1931, as noted previously, were unfavorable for the maximum development of brown spot. The varieties did not rank the same in the order of their susceptibility during both years. For example, in 1930 Mizelle and Tisdale stood at the bottom and top of the list, respectively, whereas in 1931 their rank was reversed.

TABLE 7.—*Comparative susceptibility of 10 commercial varieties of corn to Physoderma zeae-maydis in the field*

Variety	Percentage of brown spot		Variety	Percentage of brown spot	
	1930 <sup>a</sup>	1931 <sup>b</sup>		1930 <sup>a</sup>	1931 <sup>b</sup>
Mizelle.....	78.2	14	Wilson.....	46.8	28
Florida 191.....	67	20	Lowmans.....	44.1	36
Snowflake.....	63.5	42	Kilgore Red Cob Prolific.....	38.9	30
Dubose.....	53.4	40	Cuban Yellow Flint.....	36.8	38
Whitley.....	53.2	26	Tisdale.....	33.3	46

<sup>a</sup> Percentages based on 77 to 101 plants of each variety.

<sup>b</sup> Percentages based on 56 plants of each variety.

<sup>11</sup> TISDALE, W. H. Op. cit.

## COMPARATIVE SUSCEPTIBILITY OF SELF-FERTILIZED LINES OF CORN TO BROWN SPOT

Inbreeding was practiced in a number of different varieties of corn grown during epidemics of brown spot the three seasons, 1929 to 1931, to develop inbred lines for the purpose of selecting strains to be used in a study of the inheritance of the disease and in the creation of resistant synthetic varieties. The disease was induced by filling the upper whorl of leaves of the plants when 3 to 4 feet in height with a sporangial suspension of *Physoderma zeae-maydis*. Although the work has been in progress only three years, the results show that it is possible to obtain resistant and susceptible strains. Several hundred inbred lines have been developed and their reaction to brown spot studied. Of the 10 inbred lines, whose reaction to brown spot is given in Table 8, the first 5 strains were developed by selecting and self-fertilizing plants resistant to the disease, while the last 5 were developed without such careful selection.

TABLE 8.—Comparative susceptibility of 10 inbred lines of corn to *Physoderma zeae-maydis* as determined by natural and artificial inoculation in the field

Variety and inbred line number	Percentage of plants with brown spot <sup>a</sup>			Variety and inbred line number	Percentage of plants with brown spot <sup>a</sup>		
	1929	1930	1931		1929	1930	1931
Petree 8-5-4-1.....	52.9	23.0	8.6	Cuban Yellow Flint × Florida Flint 18-3-8-4.....	100.0	100.0	83.9
Early Yellow Dent 14-6-6-19.....	90.6	41.3	21.0	Cuban Yellow Flint × Florida Flint 18-2-10-17.....	62.8	82.6	90.0
Cuban Yellow Flint × Florida Flint 18-2-8-18.....	62.8	45.9	9.0	C. H. × F. B.-U. S. D. A. 73-2-9-29 (Richey).....	76.6	85.7	100.0
Wilson 57-36-2-4-10.....	44.0	37.1	12.5	Laguna 81-1-3-21.....	52.6	65.6	80.6
Laguna 81-3-2-11.....	43.7	20.0	4.0				
Mathers 12-2-2-8.....	69.7	34.4	78.8				

<sup>a</sup> Nineteen to 95 plants of each line were grown each year.

## SUMMARY

All subspecies of corn proved to be susceptible to brown spot when artificially inoculated.

Brown spot was found to be most often located on the leaf blades and sheaths of corn plants at or below the fourth node from the ground.

The most effective method of producing brown spot in the greenhouse was to inject a water suspension of sporangia into the stalks of the plants, 2 to 3 feet in height, at or near their growing points. In the field the best method of inoculation was to fill the upper whorl of leaves with inoculum from a hand pressure sprayer when the plants were 45 to 52 days of age and 35 to 51 inches in height.

The reaction of inbred lines to brown spot in the greenhouse was not a true indication of their reaction under field conditions.

Infection was produced by sporangia which had been kept in soil in test tubes buried a foot in the ground for three years, in a bottle in the laboratory for three years, in leaf sheaths exposed to the weather for two years, and by sporangia taken from diseased areas in green leaf sheaths.

Eleven days from the date of inoculation was the minimum time required for sporangia to develop in plants grown in the greenhouse; in the field 16 days were required.

More brown spot developed in the wet season of 1930 than in the dry season of 1931.

Varieties of corn differed in their susceptibility to brown spot and did not rank the same in the order of their susceptibility during the years 1930 and 1931.

Inbred lines of corn have been isolated, which differed in their susceptibility to brown spot when they were exposed to this disease.



# THE DISTRIBUTION OF NITROGEN IN TOBACCO WHEN THE SUPPLIES OF NITROGEN AND OF LIGHT ARE VARIED DURING THE GROWING PERIOD<sup>1</sup>

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## INTRODUCTION

With the exception of that done recently, nearly all the work relating to the intermediate products of plant metabolism has had its inception in a desire to isolate products for use in industry, or for therapeutic application. These compounds were for the most part by-products of certain plants and not products that are universally found in all seed plants; and they had no relation to fundamental physiological activities. The general types of compounds were alkaloids, glucosides, and certain dyes. When it became known that there is close interrelationship between ions absorbed from the nutrient medium and the amounts of organic materials synthesized, the quest for an explanation of the intermediate processes began.

The limitations of analytical chemistry are such that little information can be obtained by separating the nitrogen components of a single plant. However, plants grown under different conditions of environment should yield results capable of interpretation. The wider the variation in conditions of growth and development the greater should be the ease of correlation.

Certain root diseases of tobacco have suggested the possibility that reductase activity in the diseased plants may be less than that in normal plants. If any appreciable reduction of nitrates occurs in the root hairs, it may be due to retardation in the normal elaboration of nitrogen rather than to nitrogen deficiency or incapacity of the plant to absorb the ion. In the present experiments an attempt was made to determine the approximate location within the plant at which reduction takes place, and the effects of light and of the relative supply of nitrates on the amounts of different nitrogen fractions in the various plant parts.

## METHODS

Havana seed tobacco seedlings were planted singly in glass jars. Each plant was held in place by having its roots inserted through a hole in a cork, and cotton placed about the plant stem. The composition of the medium was as follows:

	Mol.
CaH(PO <sub>3</sub> ) <sub>2</sub> ·2H <sub>2</sub> O-----	0. 0025
CaSO <sub>4</sub> ·2H <sub>2</sub> O-----	. 0026
MgSO <sub>4</sub> ·7H <sub>2</sub> O-----	. 0057
KCl-----	. 0037
NaNO <sub>3</sub> -----	. 0278

In addition, the medium contained 3 parts per million of boric acid and a trace of ferric potassium tartrate. The media were renewed

<sup>1</sup> Received for publication June 13, 1932; issued March, 1933. Contribution No. 142 of the Massachusetts Agricultural Experiment Station.

every two days. The nitrogen content of the medium as compared with the content of other essential elements was therefore relatively high. This condition was conducive to a rapid growth of plants, characterized by little fiber and much water, and a consequent tenderness and succulence of plant parts.

The concentration of this medium, except for the nitrogen, is lower than that of most standard nutrient solutions. Preliminary experiments indicated that by renewing the media on alternate days good growth was afforded during the time these plants were growing. The experiments by Stiles (26)<sup>2</sup> would suggest that lower total concentrations may be used satisfactorily if the plant is provided with salts at more frequent intervals.

In the greenhouse 206 tobacco seedlings were planted in as many jars on May 4, 1931. The plants were allowed to grow in the greenhouse until June 4. At that time they were removed, and 100 were placed in a light room (where the light was of natural intensity and duration), and 100 were placed in a dark room. Each of these two groups of plants was divided into two groups. Fifty of the plants in the light room and fifty in the dark room continued to receive the same nutrient medium. From the other 50 in each room, sodium nitrate was entirely withheld, but the concentration of the other salts was not altered. During the 11 days of the test the temperature varied from 20° to 21° C.

Both groups of plants kept in the light continued to grow normally, whereas the two groups kept in the dark room showed definite signs of etiolation after two days. At the end of 11 days the plants kept in the light showed no unnatural symptoms, while those kept in the dark were not only markedly etiolated but also had lost a few of their lower leaves. The general appearance and flaccid texture of some of the roots of the plants kept in the dark indicated deterioration. At this time all the plants were harvested, and each plant in the four groups was divided arbitrarily into (1) roots (2) stems (3) midvein of leaves, and (4) part of the leaf left after the removal of midvein. Each of the 16 lots of plant material was sealed in a glass jar, placed in a temperature of -9.5° C. and kept frozen at this temperature until removed for analysis.

#### EXTRACTION

Nightingale, Robbins, and Schermerhorn (19) obtained concordant results in the analyses of frozen plant parts and the corresponding fresh materials. Chibnall (7) found that drying plant tissue at 40°-50° C. entailed a loss of protein and an increase in ammonia due to autolysis. Thomas (27) dried materials at 70° as the preliminary step in analysis. Totttingham, Schulz, and Lepkovsky (30) extracted considerably more soluble nitrogen and soluble protein nitrogen from frozen leaves than from the dried forms, but less from the frozen materials than from the fresh materials.

For analysis the total weight of each lot of material was determined, after which small portions were removed for dry-weight determination. These samples were dried at 105° C. in an electric oven for 36 hours. It would have been preferable to use a lower temperature in partial vacuum. However, the percentages of nitrogen fractions of a

<sup>2</sup>Reference is made by number (*italic*) to Literature Cited, p. 264.

sample dried at 105° differed only slightly from the percentages of a sample which had been dried at 100°. Portions of each lot were removed for total-nitrogen determination. The remainder of the frozen material was ground through a Nixtamal mill. The solid portions of this ground material were ground again through another mill, and in the case of the more fibrous materials, were ground one or more additional times. With water added, the solute and materials in a colloidal state were expressed through cloth. The residue was then placed in a mortar with water and ground with quartz sand, after which the materials were again placed on the cloth and the extraction repeated. Before the first grinding, no water was added. However, water was liberated from the plants, which rapidly began to thaw. After the first grinding, water was added for grinding in the mill and mortar in amounts depending on the amount of material present. The solute was eventually made up to 2,500–3,000 c c.

The residual pulp was analyzed for total nitrogen. The percentage thus obtained was compared with that obtained by subtracting the total water-soluble nitrogen from the total nitrogen.

The amount of protein that could be dissolved out of the residue (after water extraction had been employed) by heating with a 5 per cent sodium chloride solution for one-half hour at 60° C. was usually very small. This would indicate that globulins had been removed in previous extraction from all ruptured cells, or that this type of protein was present only in minute quantities. Slightly more than 0.01 per cent was extracted by means of salt water from the different plant parts.

#### TOTAL NITROGEN

Ranker (23) found the salicylic acid-thiosulphate method for the determination of total nitrogen unsatisfactory where nitrates are present. The writer found that a loss of nitrogen occurred when this method was employed in these experiments.

The method finally adopted was that in which iron is employed for reduction: The procedure was according to that outlined by Pucher, Leavenworth, and Vickery (21). A blank test was made to determine the purity of the iron.

#### WATER-SOLUBLE PROTEIN

A 200 c c portion of the extract was treated with Stutzer's reagent and allowed to stand overnight and filtered. The material on the filter was digested in the usual manner and the nitrogen determined by the Kjeldahl-Gunning-Arnold method.

#### AMMONIA AND OTHER VOLATILE BASES

A 100 c c portion of extract was made alkaline with calcium oxide, and distilled with steam. The distillate was taken up by a 0.02 N  $\text{H}_2\text{SO}_4$  solution, and this in turn was titrated with 0.02 N NaOH. Magnesium oxide was found to serve as well as calcium oxide. The use of MgO caused frothing in the subsequent determination of nitrate nitrogen. In the present instance basic nitrogen was not separated from ammonia. However, a trial indicated that the alkaloid components were small in amount at this stage of growth.

## NITRATE NITROGEN

Various methods and modifications have been suggested for determining nitrate nitrogen in the presence of organic matter. Gallagher (12) objected to the reduction of nitrates in acid solution when amino acids are present, as the intermediate compound, nitrous acid, acts to liberate nitrogen. He preferred the Valmari-Devardo method. Pyne (22) could not duplicate the analyses when he used the Valmari-Devardo method of Gallagher, and obtained better results by reducing the nitrate nitrogen by the use of titanious hydroxide.

Emmert (10) used sulphuric acid with the plant materials and drove the resultant distillate over into a flask containing chlorine dioxide. From this point he used for determining nitrate nitrogen in the distillate that part of the method suggested by Harper (14) for soil nitrates, namely, phenol disulphonic acid.

Burrell and Phillips (5) used the phenol disulphonic acid method by first eliminating the objectionable carbon compounds which would otherwise have interfered with the final color comparisons.

Breckenridge (3) obtained serious error by using the zinc-iron method for reduction of nitrates in fertilizers and concluded that the method should not be adopted as an official one.

Other investigators seem to have obtained better results in the reduction of nitrates with aluminum or an alloy of aluminum in alkaline solution. Pozzi-Escot (20) used aluminum. Shedd (24) obtained favorable results with a modification of Straud's method of using Devardo alloy.

The method finally adopted by the writer was that outlined by Vickery and Pucher (31). The extract from which the volatile bases had been expelled was used. This method gave more consistent results in this particular instance than other methods involving reduction in acid or basic solutions.

## AMIDES

Vickery and Pucher (32) stated that the hydrolysis to ammonia with HCl varied considerably with the HCl concentration.  $H_2SO_4$  is preferred as an acid for hydrolysis.

A portion of the solution from which the soluble protein had been removed by Stutzer's reagent was freed of ammonia by being distilled with steam after sufficient calcium oxide had been added to make it alkaline. To the ammonia-free portion was added sulphuric acid sufficient to make a 5 per cent acid solution. This was hydrolyzed for two hours, a reflux condenser being used in the boiling process.

After being cooled, the hydrolysate was made nearly alkaline with magnesium oxide, and then sufficient sodium hydroxide solution was added to make it distinctly basic. The ammonia was distilled by steam distillation into an acid solution 0.02 N, and titrated with 0.02 N NaOH.

## HUMIN

The solution that remained after the amide nitrogen had been removed as ammonia was filtered while hot, and the residue on the filter paper was washed. The nitrogen of this residue was then determined by the method employed for protein nitrogen.



## PROTEOSE NITROGEN

An aliquot portion of extract from which water-soluble protein had been removed was saturated with zinc sulphate after having been made slightly acid with sulphuric acid. The precipitate was allowed to stand overnight and filtered. The nitrogen of this precipitate was then determined by the method employed for protein.

## ALPHA-AMINO NITROGEN

The humin filtrate was acidified with acetic acid and the amino acid nitrogen determined by the Van Slyke method. In instances where extreme dilution was necessary, this value was very low. In such instances, ammonia was removed from the original extract and an aliquot portion used for determination.

TABLE 1.—*Dry matter and nitrogen in tobacco plants as affected by exposure to light and by fertilization with sodium nitrate*

[All computations made on dry-weight basis]

GROWN FOR 1 MONTH AND 11 DAYS IN LIGHT,  $\text{NaNO}_3$  SUPPLIED DURING THE ENTIRE PERIOD

Part of plant	Dry matter		Total N	Soluble protein N	Total water soluble N	Ammonia N	Humin N	Proteose N	Alpha-amino acid N	Amide N	Nitrate N
	Grams	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Leaves without mid-vein.....	60.258	7.260	3.861	1.091	3.810	0.123	0.003	0.076	0.967	0.464	1.090
Midvein of leaf.....	16.573	4.950	3.838	.691	3.790	.135	(*)	.380	.517	.518	1.482
Stem.....	24.365	4.419	4.963	1.114	.....	.296	.233	.662	.810	.408	.817
Root.....	16.863	5.236	3.173	.891	2.872	.230	.194	.171	.406	.297	.243

GROWN FOR 1 MONTH AND 11 DAYS IN LIGHT,  $\text{NaNO}_3$  SUPPLIED FOR 1 MONTH AND WITHHELD FOR THE LAST 11 DAYS

Leaves without mid-vein.....	51.411	6.869	3.661	1.070	3.642	0.227	0.348	0.135	0.951	0.286	0.569
Midvein of leaf.....	19.142	4.689	3.236	.669	.....	.190	.001	.142	.978	.295	.882
Stem.....	13.884	4.436	5.158	.745	.....	.391	.355	.387	.949	.774	.733
Root.....	16.649	6.017	2.382	.586	.....	.237	.079	.229	.787	.114	.194

GROWN FOR 1 MONTH IN LIGHT AND THE LAST 11 DAYS IN DARKNESS,  $\text{NaNO}_3$  SUPPLIED DURING THE ENTIRE PERIOD

Leaves without mid-vein.....	35.784	7.749	4.823	1.191	.....	0.363	0.095	0.130	0.569	0.573	1.586
Midvein of leaf.....	19.292	6.797	4.137	.377	3.758	.251	.272	.062	.813	.242	1.830
Stem.....	24.974	5.286	5.645	.775	5.529	.674	.085	.154	1.635	.454	1.311
Root.....	23.308	6.343	2.791	.691	2.288	.381	.064	.176	.459	.085	.308

GROWN FOR 1 MONTH IN LIGHT AND THE LAST 11 DAYS IN DARKNESS,  $\text{NaNO}_3$  SUPPLIED FOR 1 MONTH AND WITHHELD FOR THE LAST 11 DAYS

Leaves without mid-vein.....	34.310	8.407	5.753	0.911	5.372	0.404	0.007	0.324	1.181	0.280	1.168
Midvein of leaf.....	13.620	4.766	5.092	1.148	4.701	.532	.227	.308	1.683	.472	1.812
Stem.....	12.646	3.954	5.177	.801	5.024	.909	.198	.023	1.197	.609	1.103
Root.....	12.818	5.695	3.242	.843	2.877	.513	.069	.012	.727	.054	.121

\* Trace.

## RESULTS AND DISCUSSION

Since the plants had grown in a medium supplying an abundance of nitrate nitrogen as compared with the amounts of other plant nutrients, it would appear that during the time the plants were in the light more than the normal amount of nitrogen would be taken up (Tottingham and Lowsma (29)). This would probably be followed by a large water intake. It will be noted from Table 1 that some of the plants which had been kept in the dark for 11 days before being harvested showed a slightly higher percentage of dry matter of leaves (without midvein) than did those remaining in the light during the entire growing period. This was due in part to the fact that some of the lower leaves had died as a result of the unfavorable environment.

Smith (25) found that several varieties of plants thrive economically best when from 10 to 20 parts per million of nitrate nitrogen are present. This amount was relatively small as compared with the amount used in the present trials, in which obviously more nitrate nitrogen than was necessary was present. Haas (13), however, found that 500 parts per million of nitrate gave good results for tobacco growth and that sodium nitrate afforded conditions better for intake of  $\text{Ca}^{++}$  than the nitrate of calcium, magnesium, potassium, or ammonia. However, the literature on this subject does not justify a conclusion at this time.

The presence of some salts containing essential elements affects the intake and elaboration of nitrogen compounds. Thus Burrell (4) found that a deficiency of magnesium caused a lowering of the quantity of soluble and insoluble nitrogen in soybean leaves and stems. A deficiency of calcium also caused a lowering of both soluble and insoluble nitrogen, while potassium deficiency caused a lowering of insoluble nitrogen but little difference in soluble nitrogen.

## TOTAL NITROGEN

In these experiments under every condition the minimum percentage of total nitrogen was in the roots. To a degree this is in accord with the work of Kraus and Kraybill (16), who found that in the stem of the tomato plant there is a descending gradient of total nitrogen. In the light of recent investigations it would be well to consider how nitrogen compounds may be transported in certain plants by means other than those commonly recognized. Maskell and Mason (17) found that total nitrogen, protein nitrogen, or amino acid nitrogen may be associated with longitudinal movement, and suggest that the presence of sieve pores in cotton plants should allow the movement not only of crystalloids but also of materials in a colloidal state. The stems of these tobacco plants contained the highest percentage of total nitrogen as compared with that of other plant parts. The total nitrogen percentage of the plants grown in the dark was slightly higher, but the total quantity of nitrogen in the plants grown in the light was slightly greater on account of the increase in growth. The leaf (minus midvein) came next in order in percentage of total nitrogen, with the exception of that of the plants growing in the dark for 11 days without a supply of nitrogen. In that instance the leaf contained more than did any other one part of the plant. These plants showed abnormalities, not only in appearance but in content of nitrogen fractions.

## SOLUBLE PROTEIN

At an early stage of development of tobacco the soluble protein compounds seem to be almost equally abundant in the leaves and stem, but less abundant in the roots and veins.

The protein here described probably includes not only albumin but globulins or prolamines, etc., which might be present in minute amounts. Although the last two classes are regarded as not soluble in water, the method of extraction with water by pressing through cloth would not necessarily prevent the insoluble compounds in a fine state of division from being a part of the extract.

## AMMONIA NITROGEN AND AMIDES

In all instances the largest percentage of ammonia nitrogen was found in the stem.

In the case of amides no relationship could be shown between the amide content of the plant parts and the different conditions to which the plants were subjected, although the higher percentages were always confined to the aerial portions of the plant. No evidence was found to show that amides may be used for protein synthesis when amino acids are not present in the required amount. The fact that this condition was not arrived at was due to the presence of a constant supply of alpha amino acids during the growing period. It might be mentioned in this connection that amides have been regarded by some workers as parts which form proteins when there is a scarcity of amino acids as in cases of pronounced etiolation (6; 15, p. 247).

The conditions referred to, prevailed when amide nitrogen was the product of metabolism. On the other hand, when asparagine was used as a source of nitrogen in a growing medium for tobacco under sterile conditions, the plants reported on by Beaumont, Larsinos, Piekenbrock, and Nelson (2) obtained fair growth, though not entirely normal.

Ammonia has been regarded both as a primary and end product in nitrogen metabolism of plants.

## ALPHA AMINO ACIDS

Amino acid accumulation in darkness, as indicated by Table 1, is probably due not to more active reductase activity but rather to a piling-up effect incident to the slowing up of processes having to do with the formation of more complex nitrogen compounds. The other explanation would be that tobacco plants, which at this growing stage are sensitive to total absence of light, begin soon to react in the dark to break down proteins and use a nonnitrogenous part of the cleavage product to conserve the carbohydrate supply. The nitrogenous portion might thus eventually appear as alpha amino acids. The latter suggestion seems conjectural in view of the fact that the plant contains relatively little glucoprotein.

## NITRATES

Eckerson (8) found that in the tomato plant reduction took place at the stem tip just behind the growing region in the leaf cells, especially near the phloem and in the cortical cells of the petioles; in the stem, especially near the nodes, in the phloem parenchyma and the cells in the cortex near the phloem.

In contrast to these annuals, in some plants which have a more or less developed carbohydrate storage system, much of the nitrate nitrogen seems to be reduced at the roots. The work of Eckerson (9) and of Thomas (28) would indicate that in the apple reductase activity was more pronounced in the roots than in the aerial portion. Also, Nightingale and Robbins (18) found that apparently the nitrates are reduced to nitrites and ammonia in the roots of narcissus, with accompanied decrease of carbohydrates in these plant parts.

The outstanding feature of the analytical data is the relatively large percentage of nitrate nitrogen in the aerial portions of the plant in instances where sodium nitrate was supplied during the entire growing period. If the nitrate content of plants grown under favorable economic field conditions is compared with the nitrate content of any of these plants, it will be seen that in none of these was there nitrogen deficiency. This high percentage reaches a maximum of slightly more than 1.8 per cent in the midvein of leaves of plants grown in the dark during the last 11 days before being harvested. The maximum of nitrate nitrogen was found in the mid-rib of the leaf, as compared with the amounts in other parts of leaf, stem, and root. This was true for plants grown in the light and in the dark and regardless of whether the nitrates were withdrawn or continuously supplied. The plants seemed to have taken up a sufficient nitrate supply previous to the 11 days, when nitrogen was withheld from one-half of the number to maintain apparent normal metabolism during this time, providing normal light was retained. It would have been desirable to continue growing the plants in the light until the group no longer receiving nitrogen had nearly, or totally exhausted its accumulated supply of nitrates. If all plants were to be harvested at the same time, however, this could not be done, inasmuch as the plants growing in the dark were intolerant of a more prolonged period in the dark.

Anderson (1) found with a variety of plants that the nitrate content varies greatly according to the immediate environment, such as locality of growth, degree of shade, nitrate supply of soil, month of year, and hour of day. These factors finally resolve themselves into the supply of nitrates and the rate of anabolism.

Frear (11) found that the small leaves of beet roots contained more nitrate nitrogen than the larger ones, and that the midrib contained always more than the rest of the leaf.

In the experiments with these tobacco plants the leaf portion (other than midrib) decreased in nitrate content to a greater degree than other plant parts as a result of the withdrawal of nitrate from the medium, regardless of light conditions. The next in order of decrease in nitrate content are roots, stems, and midvein. It must be emphasized, however, that there was no evidence of nitrogen starvation, as unelaborated nitrogen was found in all plant parts. The nitrate content of plants grown in the dark showed a marked increase. This would usually be explained as being due to the fact that the gradient of photosynthesis did not keep pace with the rate of respiration, owing to carbohydrate deficiency.

The evidence at hand would seem to support the conclusion that much of the nitrate reduction in tobacco takes place in or near the veins of the leaf.

## TOTAL DRY WEIGHT

Under the most favorable conditions of the experiment, when plants were supplied for the entire growing period with nitrates and light, the total dry weight was the highest. There was little difference in the total dry weight of the plants growing during the entire period in the light, from which nitrogen had been withheld during the last 11 days, and that of those to which nitrogen had been supplied during the entire growing period but which had been held in darkness during the last 11 days. The minimum total dry weight was that of the plants from which both light and nitrates had been withheld during the last 11 days. The total dry weights in grams of the groups, in the order in which they have been described, were as follows: 118.059, 101.086, 101.358, and 73.394.

The amount of dry matter in the roots and stems of the plants from which nitrogen had been withheld was materially lower than that in the plants grown under similar conditions of light but receiving a constant supply of nitrogen. This suggests that nitrogen deficiency first affects the root and stem systems. The amount of dry matter of the entire leaf portion of the plants from which light had been withheld was lower than that of plants grown under similar condition of nitrate supply but receiving a constant supply of light. The absence of light first exerted a deleterious effect on the leaf.

## SUMMARY

Tobacco plants were grown in the greenhouse in water cultures for one month. At the expiration of this time one-half of the plants were placed in the dark and one-half were continued in the light (in light of natural duration and intensity). Sodium nitrate was continuously supplied to one-half of each of the two groups; from the other one-half of each group nitrate nitrogen was withheld for 11 days before the plants were harvested.

The plants were divided into four plant parts (roots, stems, mid-vein of leaf, and leaf other than midvein) and analyzed for various nitrogen fractions.

The stems were relatively richer in total nitrogen and ammonia than the other plant parts. The leaves were characterized by a relatively high content of dry matter and protein.

Plants deprived of a nitrogen supply for 11 days still maintained an ample supply of nitrate nitrogen in the tissue.

The plants grown in the dark for 11 days contained a higher percentage of nitrate nitrogen than the corresponding plants grown in the light.

The plants grown in a liberal supply of nitrate nitrogen, with a disproportionately low supply of other plant nutrients, contained a low percentage of dry matter and a high percentage of nitrate nitrogen, indicating a low metabolic rate. These factors did not apparently interfere with luxuriant growth at this stage of the plant's development.

The nitrate and alpha amino acid nitrogen content of the plants seemed to be most influenced by light. Darkness in most instances increased the percentage of nitrogen.

In all instances the maximum percentage of nitrate nitrogen was found in the midvein of the leaf. This would suggest that considerable reduction of nitrogen occurred in or near the veins of the leaf.

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# STUDIES ON THE DISSOCIATION OF THE BRUCELLA GROUP<sup>1</sup>

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## INTRODUCTION

Since the bovine and porcine strains of *Brucella* have been demonstrated to have pathogenicity for man, new interest in this group of organisms has resulted in a rather extensive and inclusive study of these organisms. A number of methods have been developed by which these organisms may be classified, according to source, into three groups; namely, caprine, bovine, and porcine types. The methods have not always given the same results in the hands of various workers; hence the question of reliability of these procedures arises.

Unfortunately, perhaps, none of these systems of classification takes into consideration the possible rôle that dissociation might play in inducing variability of the organisms. Hadley (3)<sup>2</sup> cites numerous instances of dissociation changes in an organism, causing not only marked colonial appearance but also disturbances in physiological and antigenic properties. The possibility of such changes in members of the *Brucella* group should be seriously considered—in fact, no system of classification can be considered accurate which fails to recognize the influence of dissociative changes. The significance of dissociation in this group has been only partly studied.

## HISTORICAL REVIEW

It seems needless to review the early literature concerning the appearance of atypical or parameditensis type *Brucella* cultures. The first description of R types in *Brucella abortus* was presented by Henry (4) who demonstrated such forms by spontaneous and enforced dissociation. He describes an opaque, granular colony that gave spontaneous agglutination. In a later publication (5) he states that by agglutinin absorption tests the R types of *Br. suis* were distinct serologically from the *Br. abortus*. Marshall and Jared (10, p. 321, 323) describe R types of *Brucella* as follows:

Older colonies showing definite rough papillæ are so sharply defined as to warrant little description. They are decidedly opaque and granular, and have irregular edges. \* \* \* The R colonies used were usually larger and were distinctly more opaque than S colonies; their structure was more granular, relative to the homogeneous structure of S colonies; in confluent growth of colonies a very sharp mosaic of spindle and other zones of demarcated growth bounded by arcs appeared \* \* \* ; edges might appear irregular, although this was the exception rather than the rule; crystals, not uncommon on stock colonies, were perhaps more frequently encountered on R colonies. Growth of R colonies was more rapid, a fair growth occurring in 24 hours. The outstanding features were increased opacity and granular central structure; in exceptional cases one might definitely

<sup>1</sup> Received for publication, Apr. 2, 1932; issued March, 1933. Journal article No. 95 (n. s.) from the Michigan Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 279.

see these points macroscopically. \* \* \* Cellular morphology was not observed in great detail; the R type cells, however, appeared longer.

In a rather extended study of dissociation, Zdrodowski, Brenn, and Voskressenski (13, p. 793) describe R types as follows:

Les colonies rondes, mais moins bombées et parfois presque plates, avec des contours irréguliers; leur structure est grossière, granuleuse; la pigmentation est verdâtre. Les cultures sur gélose s'émulsionnent mal dans la solution de NaCl; leur émulsion n'est pas stable (agglutination spontanée) et fournit en général une thermo-précipitation positive d'après Burnet. Les cultures en bouillon sont en flocons et peu à peu s'éclaircissent en formant un sédiment. Le pouvoir agglutinogène est nul ou très faible. Les cultures sont avirulentes ou très faiblement virulentes pour les cobayes.

Soule (12) claims to have obtained typical R types as well as mucoid forms.

### HISTORY OF CULTURES

The cultures used in these studies were obtained from I. F. Huddleson from the central Brucella station. The history of the cultures follows:

*Brucella abortus* 1.—This culture was obtained from the Bureau of Animal Industry, United States Department of Agriculture, prior to 1915. The source and date of isolation are unknown.

*Br. abortus* 2 and 3.—These organisms were isolated from aborted fetuses in 1915 from herd A at Michigan State College.

*Br. abortus* 4.—This strain was isolated from the udder of a cow from experimental herd of Michigan State College in 1915.

*Br. abortus* 5.—This culture was in the laboratory stock cultures prior to 1915. The source and date of isolation are not known.

*Br. suis* 400.—This culture was obtained from Dr. Don M. Griswold, of the Michigan Department of Health. It was isolated from a boar's testicle. The date of isolation is not known.

*Br. suis* 401 and 402.—These cultures were obtained from Purdue University. Culture 401 was isolated in 1925. The source and date of isolation of culture 402 are unknown.

*Br. suis* 404 and 405.—These cultures were obtained from J. W. Connaway, of the University of Missouri, in 1922. Culture 404 was isolated from a premature fetal pig from a naturally infected sow in 1922. Strain 405 was isolated from swine. The date of isolation is unknown.

*Br. suis* 408.—This strain was obtained from Professor Good, of the University of Kentucky. It was isolated from a hog. The date of isolation is unknown.

*Br. melitensis* 301.—This organism was isolated by J. P. Torrey. The date of isolation is not known.

*Br. melitensis* 312, 315, and 316.—These cultures were obtained from Doctor Burnet, of the Pasteur Institute, Tunis, Algeria. They were isolated from cases of undulant fever. Culture 316 was isolated in 1929.

*Br. melitensis* No. 318.—This organism was received from K. F. Meyer, of the William Hooper Foundation of the University of California. The source and date of isolation are not known.

### EXPERIMENTAL PROCEDURE

#### BACTERIOLOGICAL STUDY OF THE CULTURES

Prior to the dissociation studies presented in this paper, all the cultures used were plated repeatedly to eliminate all possible contamination and to reduce the possibility of mixed strains in the same culture. The pure-line strains thus obtained were then studied culturally, physiologically, and serologically to check their identity as to type. Carbohydrate fermentation tests were made on glucose, mannitol, maltose, sucrose, and lactose. Serological tests were made by test-tube agglutination method using a positive *Brucella abortus*

serum. Smears were made on liver infusion agar to determine colony appearance. For comparative purposes, it was deemed advisable that all strains should be typical smooth forms. All cultures were classified as to species by means of dye sensitivity (Huddleson (7)) and hydrogen sulphide production (Huddleson and Abell (8)). All the strains were typed as named, and no atypical results were obtained that would indicate the presence of any contamination or mixed strains of *Brucella*.

The extreme care exercised in identifying and purifying the cultures selected for study is indicated here to show that the cultures were all pure-line strains of *Brucella*. This preliminary study was made to eliminate contamination as effectively as possible, particularly as the data presented later in this paper revealed the fact that the R types of *Brucella* were culturally, antigenically, physiologically, and morphologically different from their S prototypes.

## METHODS USED TO INDUCE MICROBIC DISSOCIATION

## EXPERIMENT 1

Growing S strains in the presence of 10 per cent S antiserum has caused dissociation in such groups as the paratyphoid, pneumococci, and spore-forming bacilli, as well as many others. Accordingly, this method of producing R types was tried. All the cultures of the *Brucella* group were seeded into 10 per cent S antiserum broth, transferred serially, and plated at intervals of 48 hours. A second series of these cultures was aged in 10 per cent S antiserum broth and plated at weekly intervals. Colonies that showed rough colonial appearance were planted on liver agar slants and studied for stability of type before they were classified as rough forms.

TABLE 1.—Dissociation changes by members of the *Brucella* group induced by growing in 10 per cent S antiserum broth

[Plated at intervals of 48 hours]

Cultures	Type of colony * at indicated time of transferring													
	April 30	May 2	May 4	May 6	May 8	May 10	May 12	May 14	May 16	May 18	May 20	May 22	May 24	May 26
<i>Br. abortus</i> :														
1.....														
2.....														
3.....														
4.....														
5.....														
<i>Br. suis</i> :														
400.....														
401.....														
402.....														
404.....														
405.....														
408.....														
<i>Br. melitensis</i> :														
307.....														
315.....														
316.....														
312.....														
318.....														

\* S=smooth type; OS=smooth-opaque type; R=rough type; —=no growth.

In the series transferred every 48 hours (Table 1) no rough forms of *Brucella abortus* were found after 18 serial transfers. An opaque granular type colony which Marshall and Jared call R forms, but which the authors designate as opaque smooths (OS), was observed in the eleventh transfer, in all five strains studied. These forms persisted throughout the experiment, with a complete disappearance of the S colonies. In the case of *Brucella suis*, only one strain (No. 408) produced an R form. This appeared after the fifth transfer. The other five strains of *Brucella suis* produced OS forms after the eleventh transfer. Two of the *Brucella melitensis* strains (Nos. 301 and 318) produced R forms after the twelfth and fifteenth transfers, respectively. The remaining three strains of *Brucella melitensis* produced OS forms after the eleventh transfer. In the series that was aged and plated at weekly intervals, similar results were obtained.

#### EXPERIMENT 2

Hadley (3) has been quite successful in inducing R forms in members of the paratyphoid group through the agency of lithium chloride. This method was followed in this experiment. Tubes containing 1 per cent solution of lithium chloride in veal broth were seeded with the various strains of *Brucella* used in the previous experiment. These cultures were transferred weekly, at which time they were plated on liver infusion agar. The experiment was carried through a period of 10 weeks. The data are presented in Table 2. The results are similar to those obtained by the use of 10 per cent S antiserum broth, except that *Brucella melitensis* 301 failed to produce R forms. All strains readily produced the OS type. In general, the use of 1 per cent lithium chloride was not so satisfactory a dissociation incitant as was the 10 per cent S antiserum broth.

TABLE 2.—Dissociation changes by members of the *Brucella* group induced by growing in 1 per cent lithium chloride broth

Cultures	Type of colony * at indicated time of transferring									
	May 6	May 13	May 20	May 28	June 6	June 11	June 18	June 25	July 2	July 9
<i>Br. abortus</i> :										
1.....			S	OS	OS	OS	OS	OS	OS	
2.....			S	OS	OS	OS	OS		OS	OS
3.....			S	OS	OS		OS		OS	OS
4.....			S	OS				OS	OS	OS
5.....			S	OS				OS	OS	OS
<i>Br. suis</i> :										
400.....						OS	OS		OS	OS
401.....	OS				OS	OS	OS		OS	OS
402.....					OS	OS	OS	OS		OS
404.....					OS		OS		OS	
405.....					OS		OS	OS	OS	OS
408.....					R	R		R	R	R
<i>Br. melitensis</i> :										
301.....					OS	OS			OS	OS
315.....					OS	OS			OS	OS
316.....					OS	OS	OS		OS	OS
312.....					OS	OS	OS	OS	OS	OS
318.....			S	S	OS	SR		R	R	R

\* S=smooth type; OS=smooth-opaque type; SR=smooth and rough type; R=rough type.

## EXPERIMENT 3

It has been the experience of many laboratory workers with *Brucella* that frequently old agar slant cultures give rise to abnormal growth. For this reason, agar slant cultures were incubated at room temperature for a period of six weeks and were then transferred to veal infusion broth. With the hope of encouraging any rough tendencies in the strains induced by aging on agar for six weeks, the veal-broth cultures were aged for a period of eight weeks. At intervals during this latter aging period, these cultures were smeared on liver infusion agar to detect any changes that might have occurred. The data are presented in Table 3. At the end of 6 weeks, 3 cultures of *Brucella abortus* (Nos. 1, 4, and 5), and 5 of the 6 strains of *Brucella suis* were rough. Only strain 401 remained S type throughout the experiment. Three of the five strains of *Brucella melitensis* became rough, while strains 312 and 315 remained S type.

TABLE 3.—Dissociation changes by members of *Brucella* group induced by agar or liver agar slant then transferred to plain broth

[Slant cultures were incubated at room temperature for six weeks]

Cultures	Type of colony * at indicated time of transferring							
	Oct. 5	Nov. 2	Nov. 9	Nov. 16	Nov. 23	Nov. 30	Dec. 6	Dec. 13
<i>Br. abortus</i> (bovine strain):								
1.....	S	S	S	S	S	R	R	R
2.....	S	S	S	S	S	S	S	S
3.....	S	S	S	S	S	S	S	S
4.....	S	S	R	R	S	S	R	R
5.....	S	S	R	R	S	S	R	R
<i>Br. suis</i> :								
401.....	S	S	S	S	S	S	S	S
400.....	S	S	S	S	S	R	R	R
402.....	S	S	S	S	S	R	R	R
404.....	S	R	R	R	S	R	R	R
405.....	S	S	S	S	S	R	R	R
408.....	S	R	R	R	S	R	R	R
<i>Br. melitensis</i> (caprine strain):								
312.....	S	S	S	S	S	S	S	S
301.....	S	S	S	S	S	R	R	R
316.....	S	R	R	R	S	S	R	R
315.....	S	S	S	S	S	S	S	S
318.....	S	S	S	S	S	R	R	R

\* S=smooth type; R=rough type.

The results obtained by this process of dissociation were quite successful, as measured by the large proportion of the cultures that became rough and the slight amount of dissociation induced by the other incitants used.

The *Brucella* group, as a whole, seems to be very difficult to dissociate, as evidenced by the data obtained, and also by further experiences of the senior writer. The difficulty of dissociating members of the *Brucella* group is quite in contrast with the ease of dissociating those of the colon-typhoid group.

Certain cultures were refractory to dissociation incitants. In fact, 2 strains of *Brucella abortus*, 1 strain of *Brucella suis*, and 2 strains of *Brucella melitensis* never gave evidence of rough colonial characteristics, unless the ability to produce the OS colony is considered as a rough type. The writers are inclined to minimize the significance of this colony as a stable rough type, because all of these

forms that were studied were decidedly transitory as was indicated by their rapid return to S types by repeated transferring on liver infusion agar and by the preponderance of the S character in colonies. The writers believe that such forms may be obtained that would be quite stable. The organism used as a vaccine by Huddleson (6) may be such an organism. It has been the belief of the senior author that these forms are not rough, as measured by colonial appearance, but are a smooth type that has lost, to a large extent, the antigenic properties of an S form with a complete loss of pathogenicity. The unsuccessful attempts to dissociate these strains seem to confirm the statement of the senior writer (Mallmann (9)) that certain S strains of the paratyphoid group were unable to dissociate into rough types.

Further work on the *Brucella* group, since the completion of these studies, indicates that most of the *Brucella* strains studied were refractory to dissociation.

## STUDIES OF R STRAINS

### COLONIAL APPEARANCE OF R CULTURES

The colonial appearance of the R cultures of *Brucella* is very similar in type to the R forms of the *Salmonella* group. The colonies of the extreme or true R forms are characterized by a very irregular contour, the edges being extremely jagged, resembling the usual soil-spore-former colony. The surface of the colonies is occasionally wrinkled, flat, and spreading, with a dull appearance. Some of the colonies have a brownish tinge, which becomes more marked with age. The rough colonies are shown in Plate 1.

### MORPHOLOGY AND STAINING REACTION OF R CULTURES

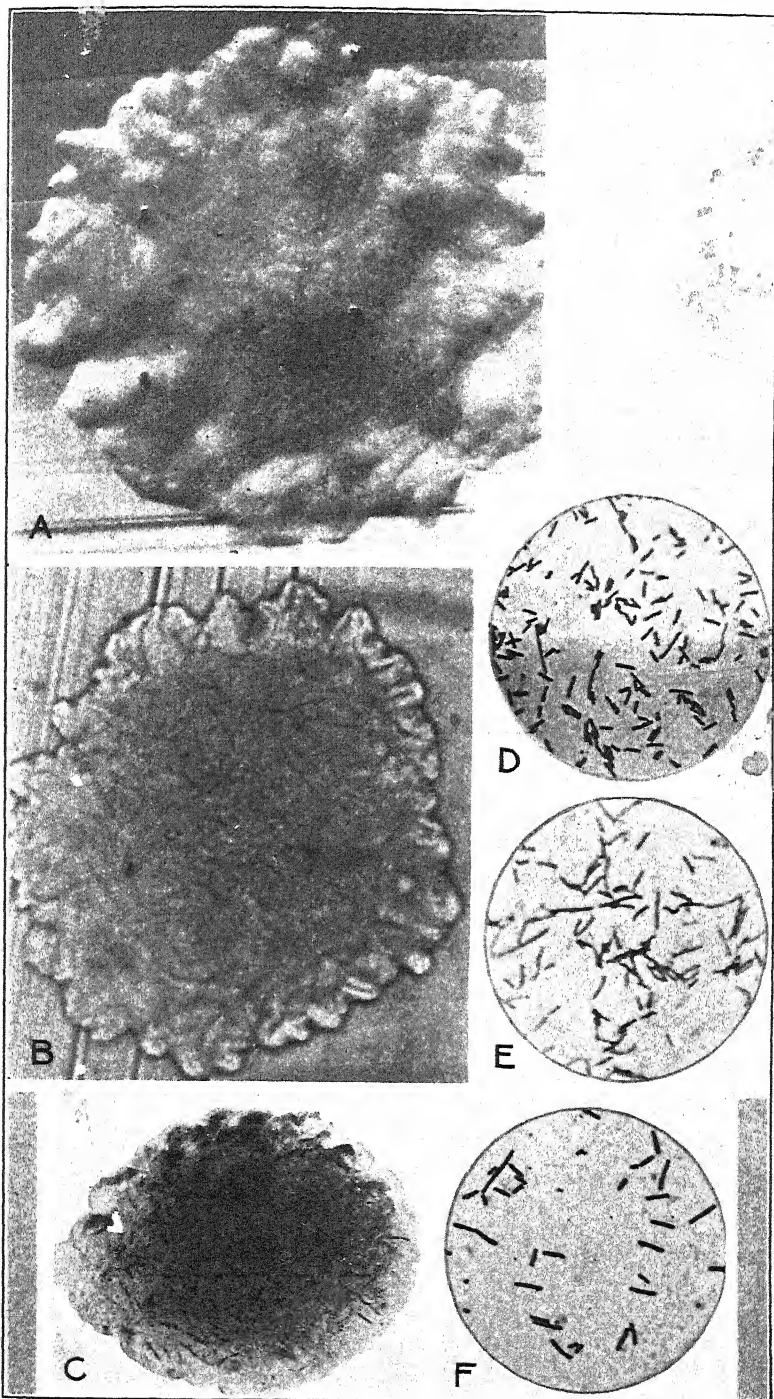
Morphologically, all the R strains of *Brucella* differed markedly from the S strains, although there was no constancy of shape and size among the R forms. The R forms were, without exception, larger than the S forms, sometimes from three to five times larger. In general, the R forms were long rods, very granular in structure, markedly pleomorphic, with a tendency to produce chains. Marshall and Jared (10) state that the R forms they studied were somewhat larger than the S forms. Photomicrographs of the R forms are also shown in Plate 1. In all instances, staining reactions with Gram's stain were similar to those of the original S forms. In fact, Gram's reaction was used as a means of identification, to the extent that all colonies giving positive Gram's stain were discarded as being probably contaminated.

### BIOCHEMICAL STUDIES OF R STRAINS

Shortly after isolation, all R strains selected for study were repeatedly plated on liver-infusion agar to determine their stability and to insure the elimination of any contamination by foreign organisms and S strains of the organism that might have been carried over in fishing the colony. The strains thus treated were then examined for their behavior on dye plates, production of hydrogen sulphide, fermentative ability, and antigenic properties.

### BEHAVIOR ON DYE PLATES

Dye plates containing thionin and basic fuchsin, respectively, were prepared according to the directions of Huddleson (7). The plates were seeded with heavy suspensions of 48 to 72 hour liver-



Rough colonies of Brucella: A, *Br. suis*, strain 402R ( $\times 30$ ); B, *Br. abortus*, strain 4R ( $\times 30$ ); C, *Br. melitensis*, strain 301R ( $\times 60$ ). Photomicrographs of B strains of Brucella: D, *Br. suis*, strain 404R ( $\times 736$ ); E, *Br. abortus*, strain 4R ( $\times 736$ ); F, *Br. melitensis*, strain 316R ( $\times 970$ )





infusion agar slant growths. The suspensions were obtained by washing the growth from agar slant cultures with small amounts of sterile broth. The seeded plates were then incubated at 37° C. for 48 hours, when the results were recorded. All strains grew equally well in both sets of plates. It will be recalled that the smooth forms of *Brucella abortus* are inhibited from growing by the action of thionin, while *Br. suis* is inhibited by the basic fuchsin. The R forms of all three species apparently had a marked tolerance for the dyes. Similar results were obtained by Marshall and Jared (10) for their opaque granular types. These experiments were repeated several times, using S forms of each species as a check, with identical results. The results indicate that the R forms could not be classified in a manner similar to that used for their S prototypes.

#### PRODUCTION OF HYDROGEN SULPHIDE

The hydrogen sulphide production was measured by the method recommended by Huddleson and Abell (8). Strips of lead acetate paper were placed inside the tubes and held in position by the cotton stoppers, the paper extending slightly below the stopper. The agar slants were heavily seeded and incubated at 37° C. The strips of paper were removed from the tubes after 24 hours and replaced with fresh ones. This procedure was repeated for seven days. The R strains of *Brucella abortus* did not produce hydrogen sulphide as did their S prototypes. The R strains of *Br. suis* produced less hydrogen sulphide than did the corresponding S strains.

The R strains of *Br. melitensis* behaved the same as the homologous S strains. The rough strains of the Brucella group, as shown by the hydrogen sulphide test, did not behave as do the S strains when typed.

#### SEROLOGICAL STUDIES OF R FORMS

A serological study of the R forms was made to determine the relationship of the various R types and to determine their antigenic relationship to their homologous S types.

Rabbits were immunized by the use of killed cultures of both R and S strains of each species. Each animal received a series of five injections given at weekly intervals. Antigens of all rough and smooth strains were made, the organism being suspended in an 0.5 per cent phenolized salt solution. Cross-agglutinations studies were made on all these antigens using the different antisera. The data are presented in Tables 4 and 5. The results show that *Brucella melitensis* and *Br. suis* R antigens were agglutinated equally well by both *Br. melitensis* and *Br. suis* R antisera, and that *Br. abortus* R antigen was not agglutinated by either of these two R antisera. As would be expected from the above results, *Br. abortus* R antiserum failed to agglutinate R antigens of *Br. melitensis* and *Br. suis*. When these R antigens were tested with S antisera, prepared by immunizing rabbits with the S prototypes of these R types, no agglutination was induced, although the S forms were all agglutinated equally well. These data, together with the data on morphology, colonial appearance, and physiological properties, emphasize the fact that the strains dealt with in this paper are R forms of a type similar to those encountered in the typhoid-paratyphoid group. They do not respond entirely to those described by Henry and by Marshall and Red.



No conclusions can be drawn from the behavior of the R strains as to their serological relations, because of the limited number of strains studied. The antigenic relationship of the R *Brucella melitensis* and R *Brucella suis* and the lack of relationship of the R *Brucella abortus* is interesting.

### REVERSION OF R FORMS

Because of the totally different characters of the R organisms studied, on the basis of their antigenic, morphological, and physiological properties, it was difficult to establish their identity as R *Brucella* until they were returned to S forms with their original characters. The methods of reversion used are presented together with the characters of the S organisms obtained.

#### REVERSION BY RAPID TRANSFERRING AND AGING

Soule (11) has demonstrated that an R type, when grown in the presence of 10 per cent R antiserum in broth, will revert to an S type. Mallmann (9) has demonstrated that the ability of an organism to revert under these conditions is dependent upon the stage or degree of roughness attained. If the organism carries a residual S character, the use of 10 per cent R antiserum in broth will induce reversion. It has been the experience of the senior writer that R types obtained by laboratory manipulation from S types can generally be reverted with little difficulty. The use of R antiserum was tried by two procedures. In the first, the culture was transferred every 48 hours in 10 per cent R antiserum broth and plated on plain liver infusion agar, and in the second, the cultures were aged in 10 per cent R antiserum broth. The results are presented in Tables 6 and 7. Strains No. 5R of *Brucella abortus* and Nos. 408R and 405R of *Brucella suis* reverted to S types in broth under the influence of rapid transferring and aging. Strain No. 405R of *Brucella suis* reverted under the influence of rapid transferring, but not by aging. Neither of the two strains of *Brucella melitensis* reverted. There was no relation between the ability of an S strain to dissociate to an R type and its ability to revert.

TABLE 6.—*Reversion of R types of Brucella induced by rapid transferring in 10 per cent R antiserum broth*

Cultures	Type of colony <sup>a</sup> at indicated time of transferring											
	Feb. 23	Feb. 25	Feb. 27	Mar. 1	Mar. 3	Mar. 5	Mar. 7	Mar. 9	Mar. 11	Mar. 13	Mar. 15	Mar. 17
<i>Br. abortus</i> :												
4R.....	R	R	R	R	R	—	R	R	—	R	R	R
5R.....	R	R	R	—	R	OS	OS	S	S	S	S	S
<i>Br. suis</i> :												
400R.....	R	R	R	R	RS	RS	—	R	R	R	—	—
402R.....	R	R	R	R	R	R	RS	R	R	R	R	R
408R.....	R	R	R	R	R	R	OS	OS	S	S	S	S
404R T.....	R	R	R	R	R	R	—	R	R	R	R	R
405R.....	R	R	R	R	R	R	—	R	R	R	R	R
<i>Br. melitensis</i> :												
301R.....	R	R	R	R	R	R	—	—	R	—	R	R
306R.....	R	R	R	R	R	R	—	R	—	—	R	R

<sup>a</sup> R=rough type; S=smooth type; OS=smooth-opaque type.

TABLE 7.—*Reversion of R types of Brucella induced by aging in 10 per cent R antiserum broth*

Cultures	Type of colony <sup>a</sup> at indicated time of transferring					
	Mar. 2	Mar. 9	Mar. 16	Mar. 23	Mar. 29	Apr. 5
<i>Br. abortus</i> :						
4R	R	R	R	R	R	R
5R	R	OS	S	S	S	S
<i>Br. suis</i> :						
400R	R		R	R	R	R
402R	R	R	R	R	R	R
408R	R	OS	S	S	R	S
404R	R	R	R	R	R	R
405R	R	R	R	R	R	R
<i>Br. melitensis</i> :						
301R	R	R	R	R	R	R
316R	R	R	R	R	R	R

<sup>a</sup> R=rough type; S=smooth type; OS=smooth-opaque type.

During the course of these experiments duplicate platings were made from the 10 per cent R antiserum broth, using plain liver-infusion agar and 1-50,000 gentian violet liver-infusion agar. There appeared to be a marked tendency for the production of S forms on the gentian violet medium. However, the forms thus induced remained S type only when kept on this medium. Transfer to plain liver-infusion agar caused a reversion to the R type, demonstrating that the S forms thus obtained were pseudosmooth colonies. For this reason the results presented in the tables were taken from data secured on the plain liver-infusion agar.

#### REVERSION BY ANIMAL PASSAGE

Numerous reports in the literature show that animal inoculation of R forms frequently causes reversion when other methods fail. Griffith (2) was able to revert R forms of pneumococci to their homologous S forms by injecting the R organism, together with the killed S organisms, subcutaneously into white mice. This was confirmed by Dawson (1).

Two methods of reversion were attempted—the inoculation subcutaneously of living R cultures with the killed S prototype and the inoculation of living R cultures alone. The suspension of living cultures was prepared in the usual manner. The killed cultures were prepared by suspending the organism in 0.5 per cent phenolized 0.85 salt solution and subjected to a temperature of 56° C. for one hour. The suspensions thus prepared were plated to check sterility. Equal amounts of living and killed organisms were mixed and diluted to a turbidity of 7 mm on the Gage nephelometer. Living R suspensions were prepared in a similar manner. Guinea pigs were inoculated subcutaneously with the mixtures of killed S and living R organisms and with the living R organisms alone. A series of four injections was given each animal at weekly intervals. The guinea pigs were killed and examined six weeks after receiving the last injection.

TABLE 8.—Reversion of *R* types of *Brucella* by animal inoculation

Guinea pig No.	Material injected	Pathological changes	Isolation of culture from	Organism isolated
1	Live <i>Br. melitensis</i> 316R. Dead <i>Br. melitensis</i> 316S.	Liver, enlarged, many grayish-white foci. Spleen, numerous white foci. Testicles, normal. Lung, greenish-black foci. Kidney, normal. Point of injection, large abscess.	Liver, spleen, lung, abscess.	<i>Br. melitensis</i> (smooth).
2	Live <i>Br. suis</i> 400R. Dead <i>Br. suis</i> 400R.	Liver, normal. Spleen, white foci. Testicles, normal. Lung, greenish-black foci. Kidney, normal. Point of injection, abscess.	Spleen, lung, abscess.	<i>Br. suis</i> (smooth).
3	Live <i>Br. abortus</i> 4R. Dead <i>Br. abortus</i> 4S.	Liver, many pearly white foci. Spleen, enlarged. Kidney, normal. Lungs, normal. Left side of humerus filled with fibrinous exudate.	Liver, spleen, left axillary region.	<i>Br. abortus</i> (smooth).
4	Live <i>Br. melitensis</i> 316R.	Liver, few white foci. Spleen, normal. Lung, normal. Testicles, normal. Point of injection, small abscess. Inguinal and cervical lymph nodes, swollen.	Liver, abscess.	<i>Br. melitensis</i> (smooth).
5	Live <i>Br. suis</i> 400R.	Liver, normal. Spleen, normal. Lung, abscess at left diaphragmatic lobe. Kidney, normal. Point of injection, abscess.	Lung, abscess.	<i>Br. suis</i> (smooth).
6	Live <i>Br. abortus</i> 4R.	Liver, grayish-white foci. Spleen, enlarged, pearly white foci. Lungs, few black foci. Kidneys, normal. Inguinal and cervical lymph nodes, swollen.	Liver, spleen, lung, lymph nodes.	<i>Br. abortus</i> (smooth).

The autopsy records with the isolations are presented in Table 8. From the guinea pigs receiving the living *R* organism and the killed *S* prototype, *S* strains were isolated from the liver, lung, spleen, and abscess at the point of injection. Smooth forms were also isolated from the guinea pigs receiving living *R* organisms alone; thus, both methods caused reversion of the *R* type to the *S* type equally well. However, the pigs receiving the killed *S* organism along with the living *R* organism showed more marked lesions and a more extensive diseased condition. In no instance was a rough form recovered.

#### STUDY OF REVERTED *R* STRAINS

The reverted *R* strains obtained by animal inoculation were isolated and studied to determine their behavior as measured by the typing methods used for classifying smooth strains of *Brucella*. It was found that all the strains had reverted to their original type, as measured by the hydrogen sulphide test and by their behavior on the thionin and basic fuchsin liver agar plates. All strains agglutinated the *S* immune serums.

#### DISCUSSION

The acceptance of methods of separating the *Brucella* group into species should not be considered as final until the effects of dissociation on such a grouping have been demonstrated.

Zdrodowski and his associates (13) show that any serological classification is wholly dependent on the phase of dissociation encountered. Marshall and Jared (10) found that the R type organisms failed to classify according to Huddleson on dye plates and hydrogen sulphide production. In the work presented in this paper, the R strains of *Brucella* failed to classify according to Huddleson's methods of grouping. However, the R forms of each species gave constant results. In Marshall and Jared's studies the species themselves varied on hydrogen sulphide production. The writers are of the opinion that pure R strains are characterized by a constancy of biochemical, cultural, and antigenic properties, and that the results reported by Marshall and Jared were due to the use of intermediate or partly rough types. This view is further sustained by their descriptions of the R types used. Their colonies were similar to those which the present writers designate as OS type and which appear in the early stages of impressed dissociation. This type of colony is the classic *paramelitensis* type. The mere fact that a change in colony appearance occurs, coupled with a change in antigenic behavior, does not argue that such a colony is a rough form. In this group of organism, as well as in some other groups of bacteria, antigenic and physiological changes occur before a change in colony appearance; hence these latter characters can not be taken as a sole measure of roughness, but rather as indications of progressive change. The fact that antigenic and physiological changes take place in the *Brucella* group as dissociation progresses from the S to the R state, and that these changes are rather gradual as measured by degree of transformation, and further that the colonial shift in structure follows the antigenic and physiologic changes makes any method of classifying this group unreliable, unless it is definitely known that the organisms in question are true S type. This is particularly true of any serological classification, especially as regards the agglutinin absorption method.

The constancy and reliability of Huddleson's dye plate and hydrogen sulphide production methods of classification, as regards S type organisms, is emphasized by this study. The fact that the R types, although having physiological and biochemical actions quite different from the S type, on reversion return to the same physiological and biochemical reactions, argues for a marked constancy of characters as represented by these tests. This work thus confirms that of Huddleson. The variability of the results of other workers using these tests may perhaps be explained on the dissociative type of organism tested.

#### SUMMARY

Rough forms of *Brucella* were obtained by aging on liver-infusion agar slants, followed by further aging in nutrient veal broth.

The rough forms of *Brucella* studied were similar to pure-line rough strains of the paratyphoid group.

Classification of S forms of *Brucella* by dye plates and hydrogen sulphide production does not hold for rough forms.

Rough forms of *Brucella* were changed to S type by passage through guinea pigs.

The value of typing S forms of *Brucella* by dye plates and hydrogen sulphide is confirmed by the return of R types to their original groups by these two methods.

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# EFFECTS OF FORMALDEHYDE ON CERATOSTOMELLA FIMBRIATA AND THE SWEETPOTATO<sup>1</sup>

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## INTRODUCTION

The objective in disinfecting sweetpotatoes (*Ipomoea batatas*) is to obtain with one treatment a practical control of all major seed-borne fungous parasites including *Monilochaetes infusca* E. and H., *Fusarium batatidis* Woll., and *Ceratostomella fimbriata* (E. and H.) Elliot. This does not necessarily involve destroying these organisms when in the tissues, since diseased sweetpotatoes with the characteristic black spots are readily discarded by careful selection during the harvest and just before bedding. The destruction of spores, which are frequently abundant on the sweetpotato and germinate well in the plant bed, is of greater significance, since sweetpotatoes are sometimes contaminated with spores even when no symptoms of disease are discernible.

Mercuric chloride and formaldehyde disinfectants have been used for some time in treating sweetpotatoes immediately before bedding them to germinate new plants. Some growers claim to have obtained good control of sweetpotato diseases in general with both chemicals. Others have been disappointed, especially in the ineffectiveness of formaldehyde against *Monilochaetes infusca*<sup>2</sup> and *Ceratostomella fimbriata*. Mercuric chloride is more widely recommended, but formaldehyde is suggested as a substitute by a number of investigators<sup>3</sup> as a general disinfectant of the sweetpotato. The suggestion that formaldehyde be employed as a substitute for mercuric chloride has resulted in its use in many localities where it seems to be more readily obtained than mercuric chloride. However, as a general disinfectant for the various fungi that attack the sweetpotato, preference is given to mercuric chloride.

If formaldehyde is used as a disinfectant, 1 pint, containing 40 per cent gas, should be used in 30 gallons of water, and it is suggested that the sweetpotatoes be treated for 5<sup>3</sup> or 15 minutes.<sup>4</sup> Treatment for a period longer than 15 minutes in a solution of formaldehyde of this strength has been found to be injurious.<sup>4</sup> The sweetpotatoes should be washed after this treatment.<sup>3</sup> The strength of the formaldehyde and the length of the treatment used in disinfecting will not control black rot, the most important seed-borne disease of sweetpotatoes.

It is the purpose of this paper to give the results of a study of the various dilutions of formaldehyde used and the length of treatment necessary to control the causal fungus of black rot and to discuss the injurious effects of the chemical on the sweetpotatoes.

<sup>1</sup> Received for publication Apr. 26, 1932; issued March, 1933. Paper No. 59 of the Journal Series of the North Carolina Experiment Station.

<sup>2</sup> POOLE, R. F. A CHEMICAL CONTROL OF SWEETPOTATO SCURF. N. C. Agr. Expt. Sta. Tech. Bul. 38, 52 p., illus. 1930.

<sup>3</sup> HARTER, L. L. SWEETPOTATO DISEASES. U. S. Dept. Agr. Farmers' Bul. 714, 26 p., illus. 1916. (Superseded by Farmers' Bul. 1059, 24 p., illus. 1919.)

<sup>4</sup> TAUBENHAUS, J. J., and MANNS, T. F. THE DISEASES OF THE SWEETPOTATO AND THEIR CONTROL. Del. Agr. Expt. Sta. Bul 109, 35 p., illus. 1915.

## METHODS

The White Jersey, a sport strain of a prolific Jersey variety which produces small white sweetpotatoes, was used in this study. This variety frequently produces 15 to 30 small sweetpotatoes on each plant. They are light colored and are most desirable for determining readily the dark-brown spots produced by *Ceratostomella fimbriata*. These small sweetpotatoes were more readily handled in the experiment than larger ones would have been. All varieties of sweetpotatoes, however, are very susceptible to black rot.

Twenty-five bushels of sweetpotatoes were used in the tests. When both the sweetpotato and the fungus were tested for tolerance to the chemical, whole sweetpotatoes were used. When the tolerance of the fungus to the chemical was tested, and when the sweetpotato was exposed to the chemical less than one minute, cross sections approximately one-half inch in thickness were used.

The inoculum consisted mostly of hyaline conidia, but olive to brown conidia, ascospores, and mycelium were also present to a limited extent. Spores for the inoculations were produced in covered culture dishes on freshly cut cross sections of raw sweetpotatoes. The sections were cut and immersed immediately in a heavy spore suspension in water, drained well, and transferred to the dishes in liter amounts. The incubation period was four days, the minimum period for obtaining the maximum number of hyaline spores. In all the tests an extremely large amount of inoculum was used.

The inoculating medium was prepared by scraping spores from the sweetpotato sections and mixing them thoroughly with water. Equal quantities of the spore suspension were placed in containers of equal size, quart fruit jars or 1-gallon crocks. When whole sweetpotatoes were treated, 1-gallon and 4-gallon crocks were used which contained, respectively, one-half gallon and 2 gallons of water and both spores and formaldehyde in amounts depending on the length of treatment and the quantity of sweetpotatoes.

When the fungus and sweetpotatoes were treated simultaneously, the formaldehyde was added to the water, and after the solution had been stirred well, the sweetpotatoes were added. At the end of the designated period of treatment, they were removed and immediately rinsed with water. By this method some spores were removed but never enough to affect the results. The whole sweetpotatoes were incubated in glass-covered culture dishes for 10 days, but very accurate results were obtained by the eighth day. Through the glass covers it was possible to observe germination, which was as quickly obtained as when sweetpotatoes are bedded in sand, the practical method of securing new plants.

When the fungus was tested for tolerance to the chemical, freshly cut cross sections of the sweetpotatoes were used to check the result, since the small percentages of spores that germinated in the liquid proved an unsatisfactory means of interpreting the results. After the designated period of treatment, the chemical solution with the spores was poured over the sweetpotatoes and immediately returned to the original container. The sweetpotato sections were always rinsed in water to remove the formaldehyde.

An average temperature of 21° C. was used during the incubation period, since optimum growth of *Ceratostomella fimbriata* is produced

at that temperature.<sup>5</sup> The temperature during these tests varied between 20° and 22°. The relative humidity in the incubation containers was kept at about 100 per cent. Sterile containers were used in all tests. An occasional contamination of *Rhizopus nigricans* occurred, but only after the black-rot disease was well developed.

A commercially pure grade of formaldehyde containing 40 per cent gas was used in all the tests in this study, although in preliminary studies one containing 37 per cent gas was shown to be only slightly less effective.

Tap water from the city system was used. The reaction of this water varied between pH 7.5 and 9. This range favored the optimum growth of *Ceratosomella fimbriata*.

#### INJURIOUS EFFECTS OF FORMALDEHYDE AND WATER ON THE SWEETPOTATO

The treatment of cut sections and whole sweetpotatoes with formaldehyde was shown to have very injurious effects and also favored greater penetration of the tissues by *Ceratosomella fimbriata*. The cross sections of freshly cut tissues when washed thoroughly after treatment showed a tolerance of formaldehyde in strengths of 1-25 for 5 minutes, 1-50 for 10 minutes, 1-75 for 15 minutes, 1-100 for 20 minutes, and 1-200 for 25 minutes. Even the sections of sweetpotato treated in these dilutions for the periods of tolerance given were later found to be more susceptible to *Trichoderma*, *Rhizopus*, *Fusaria*, *Penicillium*, and other organisms than were other sections treated for the same period in water. The whole sweetpotatoes behaved in a similar manner, since the periderm tissue was not always sound even on those that appeared to be sound or at least free from prominent mechanical injury. Furthermore, the small adventitious or feed roots developing from the cambium seemed to indicate absorption of the chemical as quickly as did the freshly cut tissues of the cross sections. (Fig. 1.) After establishing their tolerance limits to the greater strengths, sweetpotatoes were immersed for 6, 9, and 17 hours in dilutions of formaldehyde in increments of 1 to 100 up to 1 to 1,000. Severe injury, resulting mostly from water damage, followed all treatments including those in which cross sections of sweetpotatoes were immersed in water, for checks. (Fig. 2.) Observations of sweetpotatoes in water-logged plant beds indicated that they are easily injured, especially before germination begins, but the injury that developed from soaking them for a few hours was not expected to be so pronounced. The sweetpotatoes germinated new plants, but these were frequently destroyed when the soft rots developed. (Fig. 3.) In 10 days after treatment some of the sweetpotatoes were soft and elastic in character but not decomposed. They gave a distinct acetic acid taste and smell. Others developed soft rots and were quickly decomposed.

As a result of the injury caused by immersing sweetpotatoes in water, they showed a distinct grayish to brown discoloration just beneath and including the periderm and finally the whole sweetpotato. (Fig. 2.) Those immersed 4, 6, 9, and 17 hours in water later germinated well, but the rapid development of *Trichoderma*,

<sup>5</sup> LAURITZEN, J. I. INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEETPOTATOES IN STORAGE. Jour. Agr. Research 33: 663-673, illus. 1926.

Rhizopus, Fusaria, and Penicillium on the injured ones destroyed both sweetpotato and young plant. (Fig. 4.)

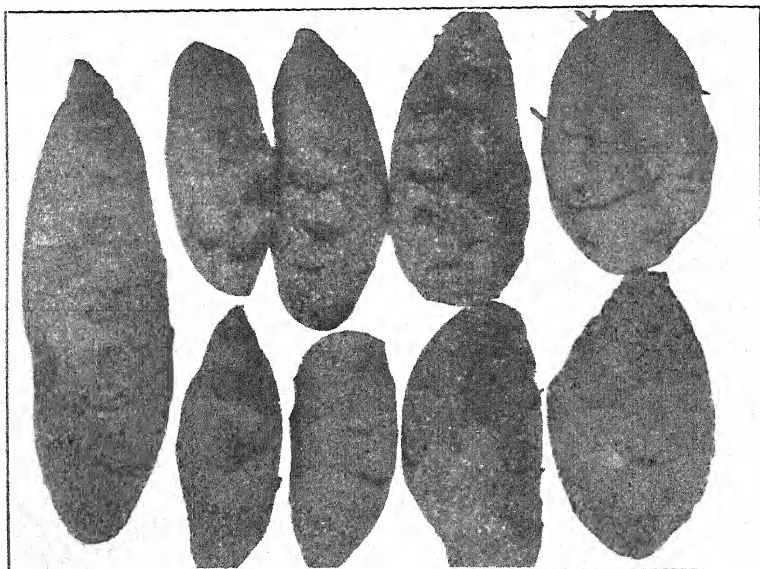


FIGURE 1.—Sweetpotatoes showing circular dehydrated spots resulting from formaldehyde injury

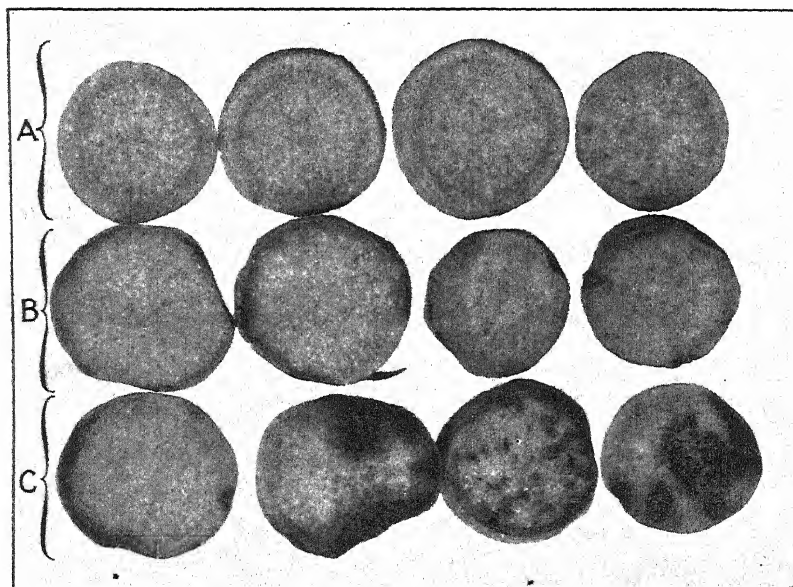


FIGURE 2.—Sections of sweetpotato: A, Healthy; B, showing breaking down, and browning and graying of tissues, especially beneath the periderm tissue, as a result of water injury; C, showing black-rot symptoms

The results of all tests clearly showed that the possibilities of a satisfactory use of formaldehyde for controlling *Ceratostomella fim-*

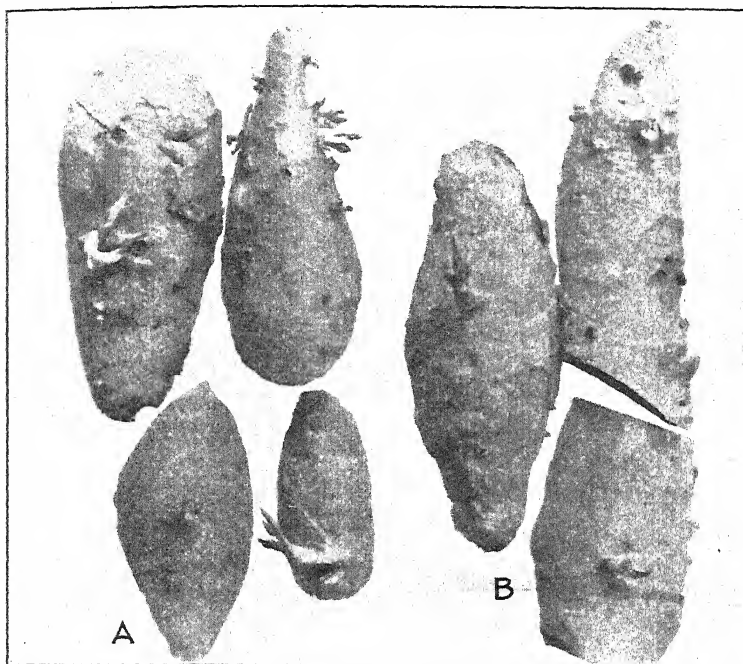


FIGURE 3.—Sweetpotatoes: A, Showing good germination after immersion in water for one minute; B, showing good germination, but with destruction of plants after the sweetpotato began to decay as a result of immersion in water for 17 hours

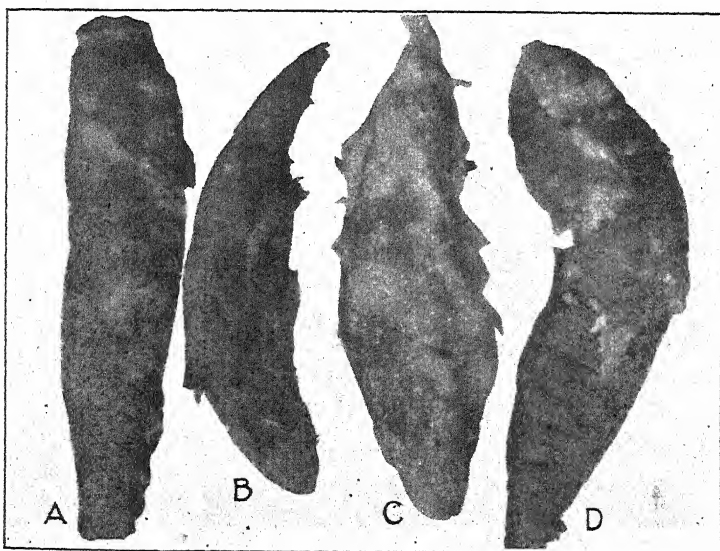


FIGURE 4.—Sweetpotatoes that were immersed in water for 17 hours and were later attacked and destroyed by: A, *Trichoderma*; B, *Rhizopus*; C, *Fusaris*; D, *Penicillium*

*briata* must be based on short periods of immersion, possible control being established in the tolerance limits of the sweetpotato to the chemical, since extended treatments with the chemical at more dilute strengths resulted in greater susceptibility of the sweetpotato to various soft rot-producing fungi.

#### CONTROL OF CERATOSTOMELLA FIMBRIATA WITH VARIOUS DILUTIONS OF FORMALDEHYDE AFTER DIFFERENT PERIODS OF TREATMENT

When formaldehyde dilutions of 1-100, 1-300, 1-500, 1-700, 1-900, 1-1,000, 1-1,200, 1-1,500, and 1-2,000 were impregnated with a heavy inoculum of hyaline and brown conidia and ascospores of *Ceratostomella fimbriata* and incubated for five days at 20° to 22° C. there was a slight germination of the fungus in the 1-1,000 and more dilute solutions, but never enough germination for accurate estimation of the kill. Furthermore, there was no germination of spores in some solutions in which heavy infection was later obtained by transferring the spores to raw sweetpotatoes. At the end of 10 days the ungerminated spores, which showed no injury microscopically, were transferred to freshly cut cross sections of sweetpotatoes in sterile fruit jars and incubated for six days. The black-rot disease developed on sweetpotatoes inoculated with spores subjected to the 1-900 and 1-1,000 dilutions.

Formaldehyde dilutions were then prepared in liter quantities beginning with 1-50, 1-75, 1-100, 1-150, and 1-200 up to 1-1,100 and were used for treating the spores for various intervals. In order to handle these cultures on a uniform time basis for completing the inoculations at the designated period, spores were added to the separate cultures at 1-minute intervals. The spores were subjected to the formaldehyde for periods of ½, 1, 4, 6, 9, and 18 hours. Sweetpotatoes were then inoculated with spores that had been treated in the cultures for the various periods. The data given in Table 1 show the effectiveness of the treatments.

TABLE 1.—Control of *Ceratostomella fimbriata* with various strengths of formaldehyde for different periods of time<sup>a</sup>

Length of treatment (hours)	Length of time (days) when fungus was observed after treatment with formaldehyde at dilution indicated <sup>a b</sup>													
	1-50	1-75	1-100	1-150	1-200	1-250	1-300	1-350	1-400	1-450	1-500	1-550	1-600	Check
½	0	9	9	4	4	4	4	4	4	4	4	4	4	4
1	0	0	9	9	9	6	9	6	6	4	4	4	4	4
4	0	0	0	0	9	9	9	6	6	6	6	6	4	4
6	0	0	0	0	0	9	9	6	9	6	6	6	6	4
9	0	0	0	0	0	0	9	6	6	6	6	6	6	4
18	0	0	0	0	0	0	0	0	0	0	0	9	9	4

<sup>a</sup> 0 indicates that no disease developed.

<sup>b</sup> 40 sweetpotato sections duplicated used in tests.

Black rot developed on the sweetpotatoes inoculated with spores taken from the formaldehyde dilutions of 1-550 to 1-1,100 after treatment for the various periods, which showed that the chemical at these strengths was not effective, even when the spores were treated 18 hours. Table 1, therefore, includes only the results obtained with dilutions of from 1-50 to 1-600 and the check. The number of days after inoculation required for the disease to develop on sweetpotatoes treated in the various dilutions for periods less than 18 hours, are

given. At the end of 12 days no disease had developed on sweetpotatoes inoculated with spores treated for one-half hour in the 1-50 dilution, for 1 hour in the 1-75 dilution, for 4 hours in the 1-100 and 1-150 dilutions, for 9 hours in the 1-250 dilution, and for 18 hours in dilutions of between 1-300 and 1-500. The "0" in this table indicates that no disease had developed at the end of the 12-day incubation period.

PERCENTAGES OF BLACK-ROT INFECTION OF SWEETPOTATOES DEVELOPING FROM SPORE INOCULUMS FROM 30 DILUTIONS OF DIFFERENT STRENGTHS AFTER DIFFERENT PERIODS OF TREATMENT

The percentages of infection given in Table 2 show the effects of the strengths of the chemical on the fungus, and the differences obtained in the infections. The results partly explain why the chemical at some of the more dilute strengths in a few instances gave less control than at those slightly stronger. There was no infection after treating the fungus one-half hour in dilutions of 1-25 and 1-50. Infections of 4.2, 13.5, and 14.2, and 100 per cent were obtained when sweetpotatoes were inoculated with spores treated for one-half hour in the 1-75, 1-100, 1-150, and 1-200 dilutions, respectively. Some spores lived in the 1-100, 1-150, and 1-200 dilutions 1 hour, and a few were not killed in the 1-200 dilution after an immersion of 4 hours. When spores were tested after treatment in the 1-250 dilution for  $\frac{1}{2}$ , 1, 4, 6, and 9 hours, the infection was 100, 15.6, 6.6, 3.2, and 0 per cent, respectively. Results of a similar character in the gradual reduction of viable spores are shown for each of the numerous treatments, which show clearly that there is a marked variation in the resistance of the spores of this fungus to formaldehyde.

TABLE 2.—Percentage of black-rot infection of sweetpotatoes developing from inoculations with spores that had been treated with various strengths of formaldehyde for different periods of time <sup>a</sup>

Formaldehyde dilution used	Percentage infection of sweetpotatoes inoculated with spores treated with formaldehyde for the period indicated					
	$\frac{1}{2}$ hour	1 hour	4 hours	6 hours	9 hours	18 hours
1-25.....	0	0	0	0	0	0
1-50.....	0	0	0	0	0	0
1-75.....	4.2	0	0	0	0	0
1-100.....	13.5	2.5	0	0	0	0
1-150.....	14.2	3.8	0	0	0	0
1-200.....	100.0	.5	.3	0	0	0
1-250.....	100.0	15.6	6.6	3.2	0	0
1-300.....	100.0	25.0	7.5	6.2	4.1	0
1-350.....	100.0	36.1	4.7	2.6	0	0
1-400.....	100.0	28.3	7.8	3.1	0	0
1-450.....	100.0	100.0	5.9	3.7	4.1	0
1-500.....	100.0	100.0	13.1	5.9	3.0	0
1-550.....	100.0	100.0	15.5	6.6	5.9	2.0
1-600.....	100.0	100.0	23.6	11.5	8.3	2.3
1-650.....	100.0	100.0	13.0	4.7	4.7	2.4
1-700.....	100.0	100.0	12.4	12.5	8.6	4.3
1-750.....	100.0	100.0	20.0	17.9	9.3	3.5
1-800.....	100.0	100.0	20.0	20.0	5.0	3.2
1-850.....	100.0	100.0	20.0	6.6	4.1	3.3
1-900.....	100.0	100.0	7.3	4.5	4.0	3.1
1-950.....	100.0	100.0	13.1	7.1	5.3	3.1
1-1,000.....	100.0	100.0	23.0	16.6	12.0	10.0
1-1,100.....	100.0	100.0	40.0	22.5	15.4	7.5
1-1,200.....	100.0	100.0	100.0	31.8	20.2	3.6
1-1,300.....	100.0	100.0	100.0	16.6	13.6	7.6
1-1,500.....	100.0	100.0	100.0	28.9	100.0	8.3
Check.....	100.0	100.0	100.0	100.0	100.0	100.0

<sup>a</sup> 40 sweetpotato sections duplicated used in tests.



The results of treatments of all spores that developed on cut sweetpotatoes three and six days after inoculation indicated that the younger spores were less resistant to the chemical than were the older spores even of the same type. However, a more scientific explanation concerning this relation must await the development of a satisfactory means of separating spores of different ages. The treatment was repeated three times, with similar results. The effects were most manifest on the margins or near points where control was almost obtained but always clearly defined as the time of treatment was increased. On the margins control was sometimes obtained in weaker dilutions, possibly because of a difference in spore resistance. In this test an infection of 4.1 per cent of diseased sweetpotatoes was obtained after spores were taken from the 1-300 dilution after a treatment of 9 hours, but no disease developed after inoculating them with spores from the 1-350 and 1-400 dilutions when the treatment was also 9 hours. An average of 40 sweetpotato sections were used, and in most instances the low percentages shown here represent a single infection. It is, therefore, possible that the fact that weaker dilutions showed greater control for the same period of treatment than did a few slightly stronger ones may have been due to the sweetpotatoes failing to pick up the few spores that were not killed. Furthermore, the few viable spores that survived after the longer treatments may also have been removed during the rinsing. The check inoculations showed uniform infection after the spores had remained 18 hours in water. Six consecutive inoculations with the same inoculum, similar in amount to that used in the treatments, showed heavy infections of equal intensity in the checks.

#### EFFECTS OF FORMALDEHYDE IN VARIOUS STRENGTHS FOR DIFFERENT PERIODS OF TREATMENT ON CERATOSTOMELLA FIMBRIATA AND THE SWEETPOTATO

Formaldehyde solutions in strengths of 1-300, 1-400, 1-500, 1-600, 1-700, 1-800, 1-900, 1-1,000 and 1-1,100 impregnated with spores of *Ceratostomella fimbriata* were prepared. Sweetpotatoes were treated in these solutions for 6, 9, and 17 hours. The spores and sweetpotatoes, therefore, were treated simultaneously for similar periods.

Formaldehyde in 1-25, 1-50, 1-75, and 1-100 dilutions was used for treating cut pieces and whole sweetpotatoes. These treatments were based on the tolerance of the sweetpotato and the fungus to the chemical as shown in previous tests. The length of the treatments was both less and greater than that shown to give control in previous tests. The results of treating whole sweetpotatoes, with the exception of other shorter and longer treatments used, are given in Table 3. The disease did not develop on sweetpotatoes when the spores were treated 14 minutes in the 1-25 dilution. There was 9.1 per cent infection after the 12-minute treatment, and even after the 1-minute treatment some kill was obtained as indicated by less than 100 per cent infection. The spores treated for 35 minutes in the 1-50 dilution developed 8.2 per cent infection but not any after treatment for 40 minutes. Black rot developed on sweetpotatoes inoculated with spores treated for 65 minutes in the 1-75 dilution, but there was no infection from spores treated for 70 minutes. The 1-100 dilution killed the spores after treatment for 100 minutes, but 18.1 per cent infection developed from spores that had been treated 90 minutes.



The number of black rot spots developing on the sweetpotatoes inoculated with spores from the various solutions tended to increase with the greater dilutions of the chemical and with the shortening of the treatment from the point at which control was obtained. The check inoculation gave a maximum of 40 black-rot spots on sweetpotatoes. There were 7 spots on the sweetpotatoes after a 1-minute treatment of both fungus and sweetpotatoes in the 1-25 dilution, 3 spots after 5 minutes, and 1 spot after 8 minutes.

Injury resulted from the use of formaldehyde at all strengths when the treatment was given over a period of time sufficient to kill the spores and persisted even when the treated sweetpotatoes were washed well in running water.

TABLE 3.—The effect on sweetpotatoes and the fungus *Ceratostomella fimbriata* of using formaldehyde of various strengths for different periods of time<sup>a</sup>

Formaldehyde dilution used	Length of treatment	Infection produced	Maximum number of spots on potatoes	Injury to sweetpotatoes	Formaldehyde dilution used	Length of treatment	Infection produced	Maximum number of spots on potatoes	Injury to sweetpotatoes
	Minutes	Per cent				Minutes	Per cent		
1-25-----	5	66.0	3	Slight.	1-75-----	45	44.4	3	Severe.
	6	50.0	2	Do.		52	44.4	3	Do.
	8	10.0	1	Moderate.		55	55.5	3	Do.
	10	12.5	1	Severe.		60	13.3	1	Very severe.
	12	9.1	1	Do.		65	8.3	1	Do.
1-50-----	14	0	0	Very severe.	1-100----	70	0	0	Do.
	10	100.0	4	Slight.		30	100.0	12	Slight.
	15	50.0	3	Do.		40	100.0	7	Do.
	20	60.0	2	Do.		52	100.0	7	Severe.
	25	11.1	2	Severe.		60	100.0	5	Do.
1-75-----	30	12.5	2	Do.	Check-----	70	60.0	2	Do.
	35	8.2	1	Very severe.		80	20.0	1	Do.
	40	0	0	Do.		90	18.1	1	Do.
	30	100.0	6	Slight.		100	0	0	Very severe.
	35	41.7	4	Severe.			100.0	40	None.
	40	60.0	4	Do.					

<sup>a</sup> 40 whole sweetpotatoes in duplicate used in tests.

## SUMMARY

A study of the effects of different strengths of formaldehyde used for various periods in treating the fungus *Ceratostomella fimbriata* (E. and H.) Elliot and the sweetpotato, shows that formaldehyde is not a practical or satisfactory disinfectant for sweetpotatoes and indicates that there is no safe strength and length of treatment that the chemical can be used for treating them.

The spores of *Ceratostomella fimbriata* were controlled in formaldehyde containing 40 per cent gas after 14 minutes in a strength of 1-25, 40 minutes in 1-50, 70 minutes in 1-75, 100 minutes in 1-100, 4 hours in 1-150, 6 hours in 1-200, and 9 hours in 1-250.

Some spores were killed even in weak dilutions and after short periods of exposure to the chemical, whereas others required a much longer treatment, indicating greater resistance for the same type of spore.

The sweetpotato was severely injured by the chemical in 1-25, 1-50, 1-75, 1-100, and 1-150 strengths even at short exposures that did not kill the spores. Treatments for four hours in 1-200, 1-250, and 1-300 dilutions did not control the fungus, and this length of treatment caused a definite breaking down of the sweetpotato and greater susceptibility to decay from other fungi.

Sweetpotatoes injured by both chemical and water were very susceptible to *Trichoderma*, *Rhizopus*, *Penicillium*, *Fusaria*, and other organisms which frequently destroyed the sweetpotato or suppressed the germination of new plants.

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## EFFICACY AND SAFETY OF ABORTION VACCINES PREPARED FROM BRUCELLA ABORTUS STRAINS OF DIFFERENT DEGREES OF VIRULENCE<sup>1</sup>

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### INTRODUCTION

A considerable amount of research has been done during the last quarter of a century to gain more definite information on the value of biologics in controlling infectious abortion. Some of the results reported have stimulated hope that biological prophylaxis may eventually be improved to such a degree that it will serve a useful purpose in the control of Bang's disease. Other results have been less favorable and in some instances have even created doubt as to whether methods of combating the disease based on artificial immunization will ever be developed to a point where confidence can be placed in them.

Favorable results in general have appeared to be confined to efforts to immunize by the use of abortion vaccines (suspensions of living *Brucella abortus*). Such vaccines, when prepared from virulent strains of *Br. abortus*, have commonly been administered only to nonpregnant animals on the theory that when subcutaneously administered to such animals the vaccinal infection would be overcome in a short time. Since they are incapable of conferring the disease, vaccines prepared from avirulent strains of *Br. abortus* have been administered to both pregnant and nonpregnant animals.

As investigations of artificial immunization progressed, it was discovered that all nonpregnant animals do not resist or promptly overcome the vaccinal infection in their bodies when virulent strains are employed in the preparation of the vaccines. This fact was indicated by agglutination tests of blood and examinations of the milk of vaccinated animals. The danger of implanting *Brucella abortus* for prolonged periods by vaccination has seemed to be greater in cows with functioning udders than in virgin heifers.

The possibility of causing an increased number of carriers of the abortion microorganism by vaccination has not been pleasing to contemplate, especially since it has been discovered that *Brucella abortus* may cause undulant fever in man.

The use of avirulent strains in the preparation of vaccines would seem to eliminate the objectionable feature. It remains to be determined, however, whether strains that have lost their disease-producing characteristics may not also have lost much of their efficacy as immunizing agents. The possibility of this has already been indicated, at least in some measure, by results of experiments made at the

<sup>1</sup> Received for publication Apr. 28, 1932; issued March, 1933.

Experiment Station in 1926 and 1928 with a small number of cattle.<sup>2</sup> It was with the object of acquiring more definite information as to the comparative immunizing value of different strains of *Br. abortus* and their freedom from objectionable effects, a feature that had previously received limited consideration in a vaccination experiment with calves,<sup>3</sup> that the experiment reported in this paper was undertaken.

#### VACCINES USED AND TESTS FOR VIRULENCE

The vaccines used in this experiment were prepared from three different *Brucella abortus* (bovine) strains, numbered 11, 19, and 484, representing three degrees of virulence. Strain 11 had been propagated on artificial culture media for 12 years; strain 19, for 6 years; and strain 484, for only 6 months.

The method used for estimating the degree of virulence possessed by each strain consisted in inoculating guinea pigs both subcutaneously and intra-abdominally with suspensions of the strains, killing the animals after about nine weeks, examining them for indications of *Brucella abortus* lesions, and making agglutination tests of their blood sera taken at the time of autopsy. Table 1 shows the results of inoculations obtained with vaccines prepared from strains 11, 19, and 484, the dosages and intervals between inoculation and autopsy being similar.

Although the guinea pigs inoculated with vaccine from strain 11 yielded reasonably marked agglutination reactions, they did not show visible lesions of *Brucella abortus* infection at the time of autopsy. The guinea pigs inoculated with vaccine from strain 19 gave more marked serological reactions and a few animals showed moderately marked *Br. abortus* lesions. All the guinea pigs which received strain 484 gave pronounced serological reactions and showed extensive *Br. abortus* lesions.

<sup>2</sup> COTTON, W. E. EFFICACY OF DIFFERENT STRAINS OF *BRUCELLA ABORTUS* AS IMMUNIZING AGENTS AGAINST INFECTIOUS ABORTION. Jour. Agr. Research 45: 705-724. 1932.

<sup>3</sup> BUCK, J. M. STUDIES OF VACCINATION DURING CALFHOOD TO PREVENT BOVINE INFECTIOUS ABORTION. Jour. Agr. Research 41: 667-689. 1930.

TABLE 1.—Guinea-pig-inoculation results with three strains of *Brucella abortus* vaccine

## STRAIN 11 VACCINE (GROWN ON CULTURE MEDIUM FOR 12 YEARS)

Guinea pig No.	Site of injection	Date of injection, 1929	Date animal was killed, 1929	Agglutination results at serum dilutions indicated *					Autopsy findings
				1:25	1:50	1:100	1:200	1:500	
7788	Abdomen	Apr. 23	July 1	+	+	+	—	—	No lesions.
7789	do	do	do	+	+	—	—	—	Do.
7790	do	do	do	+	+	—	—	—	Do.
7791	do	do	do	+	+	—	—	—	Do.
7792	do	do	do	+	+	—	—	—	Do.
7794	Subcutis	do	do	+	+	+	—	—	Do.
7795	do	do	do	+	+	+	—	—	Do.
7796	do	do	do	+	+	+	+	—	Do.
7797	do	do	do	+	+	+	—	—	Do.
7798	do	do	do	+	+	P	—	—	Do.
7799	do	do	do	+	—	—	—	—	Do.
7900	do	do	do	P	—	—	—	—	Do.
7901	do	do	do	+	+	+	+	+	Do.
7902	do	do	do	+	+	+	+	+	Do.
7903	do	do	do	+	+	—	—	—	Do.
7904	do	do	do	+	+	+	—	—	Do.
7905	do	do	do	+	+	+	—	—	Do.

## STRAIN 19 VACCINE (GROWN ON CULTURE MEDIUM FOR 6 YEARS)

7740	Abdomen	Apr. 23	June 25	+	+	+	P	—	No lesions.
7741	do	do	do	+	+	+	+	—	Do.
7742	do	do	do	+	+	+	+	—	Carpal joints slightly enlarged.
7743	do	do	do	+	+	+	P	—	No lesions.
7744	do	do	do	+	+	+	+	—	Spleen slightly enlarged; testicles atrophied.
7745	do	do	do	+	+	+	+	+	Testicles atrophied.
7758	Subcutis	do	do	+	+	+	—	—	No lesions.
7759	do	do	do	+	+	+	+	+	Do.
7760	do	do	do	+	+	+	+	S	Do.
7761	do	do	do	—	—	—	—	—	Do.
7762	do	do	do	+	S	—	—	—	Do.
7763	do	do	do	+	+	—	—	—	Do.
7764	do	do	do	+	+	+	+	+	Do.
7765	do	do	do	+	+	+	+	+	Do.
7766	do	do	do	+	+	+	—	—	Spleen enlarged.
7768	do	do	do	+	+	+	—	—	No lesions.
7769	do	do	do	+	+	+	+	+	Do.

## STRAIN 484 VACCINE (GROWN ON CULTURE MEDIUM FOR 6 MONTHS)

7783	Abdomen	Apr. 23	July 3	+	+	+	+	+	Extensive lesions.
7784	do	do	do	+	+	+	+	+	Do.
7786	do	do	do	+	+	+	+	+	Do.
7787	do	do	do	+	+	+	+	+	Do.
7906	Subcutis	do	do	+	+	+	+	+	Do.
7907	do	do	do	+	+	+	+	+	Do.
7908	do	do	do	+	+	+	+	+	Do.
7909	do	do	do	+	+	+	+	+	Do.
7910	do	do	do	+	+	+	+	+	Do.
7911	do	do	do	+	+	+	+	+	Do.
7912	do	do	do	+	+	+	+	+	Do.
7913	do	do	do	+	+	+	+	+	Do.
7914	do	do	do	+	+	+	+	+	Do.
7915	do	do	do	+	+	+	+	+	Do.
7916	do	do	do	+	+	+	+	+	Do.
7917	do	do	do	+	+	+	+	+	Do.

(\*) Key: + = complete agglutination; P = partial agglutination; S = trace of agglutination; and — = no agglutination.

## EXPERIMENT ANIMALS AND METHODS OF VACCINATION

Thirty-six cows and heifers, all of which were entirely negative to the agglutination test for Bang's disease, were used in the experiment. Three cows and six heifers received strain 11 vaccine; 3 cows and 6

heifers received strain 19; 3 cows and 6 heifers received strain 484; and 9 heifers were reserved as controls.

In the preparation of the vaccines, the strains were propagated on potato-agar slants in 18 mm tubes. The transfers were incubated for 72 hours, after which the growths were washed off the medium with physiological saline solution. Twenty cubic centimeters of saline was used for removing the growth from each tube.

To determine whether the size of dose was important in conferring immunity, different sized doses of vaccine were used. Four animals in each of the first three groups received, subcutaneously, the growth from 1 potato-agar slant suspended in 20 c c of saline. This vaccine represented a density of about ten times that of tube 1 of the McFarland nephelometer. The same number of animals received in the same manner 10 c c of this vaccine diluted with 10 c c of saline. In all cases half the dose was injected under the skin in front of each shoulder rather than at one point in order to prevent, if possible, severe swellings at the points of injection. The one remaining animal in each group of principals received intradermically one-fourth c c of vaccine of the same density as that of the undiluted vaccine which was administered subcutaneously. Each of the vaccinated animals received but a single treatment.

The vaccines were used on the day they were prepared and were proved to be viable by the inoculation of cultures and of guinea pigs.

Temperatures of the vaccinated cattle were taken just before the administration of the vaccines and on numerous days thereafter to determine whether the degree of immunity conferred bore any relation to the temperature reactions. The thermic reactions appear in Table 2.

In general the temperatures appeared to rise gradually for three days after vaccination. Then they commenced to subside and three days later were again normal.

On the first and second days after vaccination, swellings which occurred at the points of inoculation in general were small, but on the third day most of them were from 2 to 3 inches in diameter and from one-half to three-fourths inch thick. After the third day they gradually diminished in size.

The vaccine from strain 484 seemed to produce more local disturbance than that from either strain 11 or strain 19. In two animals receiving strain 484, there were indications of abscess formation at points of injection, although evacuation of abscess contents was never observed. At no time during the week after vaccination did the animals appear to be lacking in desire for feed.

Breeding was commenced two months after vaccination, but some difficulty was experienced in getting all the animals with calf. The trouble was not confined wholly to the vaccinated stock but was experienced with the controls as well. It was necessary to substitute other heifers for several of the original controls. The breeding difficulties were considered to be partly due to the fact that even during the summer months it was necessary to use a good deal of hay instead of green feed as roughage. The keeping of accurate breeding records was interfered with to some extent by the failure of the animals to show signs of oestrus regularly or always to conceive when served, for, to facilitate breeding, the bull was eventually permitted to run with the cattle during the entire day.

TABLE 2.—Temperatures of cattle immediately preceding injection and on six days after injection with three *Brucella abortus* strains

## GROUP VACCINATED WITH STRAIN 11 VACCINE (OF LOW VIRULENCE)

Animal No.	Preinjection temperature on Apr. 23	Postinjection temperature on—					
		Apr. 24	Apr. 25	Apr. 26	Apr. 27	Apr. 29	Apr. 30
Cow:	°F.	°F.	°F.	°F.	°F.	°F.	°F.
1462.....	102.6	106.2	105.0	106.2	103.0	103.0	100.6
1498.....	101.6	103.0	101.6	101.8	101.6	101.6	101.6
1500.....	102.4	104.0	101.8	105.8	103.0	101.8	101.6
Heifer:							
1441.....	102.6	105.6	105.4	105.2	103.5	103.8	102.4
1492.....	102.4	105.8	104.8	105.6	103.3	102.0	101.0
1493.....	102.0	106.0	106.2	106.2	103.8	102.5	101.6
1507.....	101.6	104.6	104.2	106.0	104.4	102.0	101.2
1533.....	103.0	106.0	106.2	106.0	104.8	103.8	100.0
1536.....	102.6	104.6	106.2	106.0	103.8	101.0	101.2

## GROUP VACCINATED WITH STRAIN 19 VACCINE (OF MEDIUM VIRULENCE)

Cow:							
1452.....	101.8	103.6	103.6	105.2	103.2	101.8	101.2
1490.....	102.2	102.8	101.6	102.0	101.8	101.6	101.4
1501.....	102.4	104.6	103.8	105.0	103.2	101.0	101.0
Heifer:							
1451.....	101.6	107.0	105.8	106.0	103.0	102.4	102.0
1495.....	102.0	104.6	104.2	105.8	104.4	101.6	101.6
1505.....	102.0	105.8	106.0	105.4	104.2	101.0	100.8
1529.....	101.8	103.6	106.0	105.8	104.4	103.2	102.6
1530.....	101.6	106.0	105.8	105.2	103.2	102.2	101.0
1537.....	103.0	105.8	105.2	106.0	104.2	102.5	102.0

## GROUP VACCINATED WITH STRAIN 484 VACCINE (OF HIGH VIRULENCE)

Cow:							
1461.....	103.5	105.8	106.0	105.6	102.2	102.2	101.6
1502.....	102.0	104.2	104.6	104.0	102.8	102.4	102.0
1504.....	102.8	104.6	104.8	105.8	101.8	101.2	102.4
Heifer:							
1356.....	103.0	106.2	106.6	106.4	105.0	103.8	102.2
1396.....	103.0	104.2	105.2	104.5	102.6	102.2	101.6
1491.....	101.8	105.8	104.8	105.8	102.8	102.2	101.0
1499.....	102.8	105.0	105.6	106.0	102.4	102.4	101.4
1528.....	102.6	106.0	106.0	105.4	102.0	102.4	102.0
1532.....	102.6	106.0	106.6	106.0	102.0	101.8	101.4

Since each group of cattle that received vaccines prepared from the different *Brucella abortus* strains contained three cows from which milk or other secretions of the udder could be obtained, steps were taken to determine whether the abortion microorganisms were eliminated from this organ after vaccination. It was planned to obtain composite samples of milk at weekly intervals and inject each of four guinea pigs intra-abdominally with 5 c c of each sample. Since none of the cows were being milked regularly it was sometimes difficult to obtain sufficient quantities of material for these inoculations. The first lot of samples was obtained one week after vaccination. The collection and testing of milk or other udder secretions were continued for more than five months.

# TESTS FOR BRUCELLA ABORTUS IN UDDER SECRETIONS OF VACCINATED ANIMALS

The guinea pigs inoculated with the udder secretions were killed approximately two months after inoculation. Agglutination tests of their blood sera and the autopsy findings furnished the information on which the presence or absence of *Brucella abortus* in the samples was based. Table 3 presents the inoculation results.

TABLE 3.—Results of guinea-pig-inoculation tests made with the milk or other udder secretions of nonpregnant cows vaccinated on April 23, 1929

## COWS VACCINATED WITH STRAIN 11 VACCINE (OF LOW VIRULENCE)

Cow from which udder secretion was obtained	Guinea-pig results on date indicated <sup>a</sup>											
	Apr. 30	May 7	May 14	May 21	May 28	June 4	June 11	June 18	July 2	Aug. 13	Sept. 21	Oct. 21
No. 1462.....	-----	-	-	-	-	-	-	-	-	-	-	-
No. 1498.....	-	-	-	+	-	-	-	+	-	-	-	-----
No. 1500.....	-	-	-	-	-	-	-	-	-	-	-	-

## COWS VACCINATED WITH STRAIN 19 VACCINE (OF MEDIUM VIRULENCE)

No. 1452.....	-----	-	-	-----	-	-----	-	-	-	-	-	-
No. 1490.....	+	+	+	+	+	+	+	+	+	+	+	+
No. 1501.....	-	-	-	-	-	-	-	-	-	-	-	-

## COWS VACCINATED WITH STRAIN 484 VACCINE (OF HIGH VIRULENCE)

No. 1461.....	-----	-----	-----	-----	-	+	-----	-----	+	+	-----	-----
No. 1502.....	-	-	-	+	+	+	+	+	+	+	+	+
No. 1504.....	-	+	+	+	+	+	+	+	+	+	+	+

<sup>a</sup> - indicates absence of *Br. abortus* infection in guinea pigs; + indicates abortion disease acquired by one or more guinea pigs.

Table 3 shows that the three cows which received the vaccine prepared from the most virulent strain, No. 484, eliminated *Br. abortus* in their milk for an extended period. Cow 1490, which received intradermically one-fourth c c of vaccine prepared from the strain of medium virulence, No. 19, also eliminated the abortion microorganism in her milk as long as these tests were continued, but no evidence was obtained that *Br. abortus* was present in the milk of the other two cows, Nos. 1452 and 1501, which received subcutaneously 10 and 20 c c of vaccine prepared from this strain. The milk of cow 1498, which received intradermically one-fourth c c of the vaccine prepared from strain 11, the least virulent of the three strains used, on two occasions gave evidence of being slightly infectious for guinea pigs. Although the injections of her milk failed to produce visible lesions in the guinea pigs, these injections produced agglutination reactions of medium intensity in some of the pigs on two different dates. In none of the tests was any evidence obtained which indicated that 20 c c of vaccine prepared from strain 11 caused the milk of the other two cows, Nos. 1462 and 1500, to contain the abortion organisms. The results of these studies strongly indicate that intradermic injections of suspensions of *Br. abortus* may result in the implantation of the infection more frequently than subcutaneous injections and would, therefore, be seriously objectionable in vaccination procedures.



EXPOSURE OF EXPERIMENT ANIMALS TO *BRUCELLA ABORTUS*

The difficulties that were encountered in getting all the vaccinated animals and controls pregnant at about the same time necessitated their exposure to virulent strains of *Brucella abortus* after pregnancy on different dates and to different strains of the infection. Final results were obtained, moreover, on but 23 animals of the vaccinated groups, whereas the number originally entered in the experiment was 27. Two animals in the strain-11 vaccine group, cow 1500 and heifer 1507, and one animal in the strain-19 vaccine group, heifer 1530, failed to become pregnant after many services. Cow 1461 in the strain-484 vaccine group died five months after vaccination. The cause of death could not be definitely determined.

Thirteen principals and five controls were subjected to *Brucella abortus* exposure on October 19, 1929; 4 principals and 1 control, on February 24, 1930; 5 principals and 1 control, on June 10, 1930; and 1 principal and 1 control, on October 22, 1930. One or more animals treated with each lot of vaccine were included among the number which received virulent *Br. abortus* exposure on each occasion except the last.

The method of exposure employed to determine the degree of immunity which the vaccine conferred was a departure from the methods commonly used in vaccine experiments. From three to four drops of a suspension of *Brucella abortus*, approximately 20 times the density of that of tube 1 of the McFarland nephelometer, was deposited on the conjunctiva of one eye of each animal. Since different animals were exposed on four different dates, it was important that suspensions of equal virulence be employed. In preparing the suspension for the first lot of animals, three strains of *Br. abortus* recently isolated from aborted fetuses were used. The suspensions represented the second, fourth, and fifth transfers, respectively, of these strains. For the second lot of animals, a single recently isolated strain of *Br. abortus* was used. This suspension was prepared from the third transfer of this strain. For the third lot, the third transfer of a single strain of *Br. abortus* was used, and for the fourth lot the second transfer of a single strain was employed.

At times of calving or abortion, six guinea pigs were inoculated with emulsions of uterine fluid or placental material that had been emulsified. Each of six guinea pigs was also inoculated with a composite sample of 5 c c of colostrum. In cases of abortion, cultural studies were also made of the fetuses. Table 4 shows the immunizing results with the cows and heifers used in this experiment. The data show that of the 23 vaccinated cows and heifers for which final records were obtained, 21 produced apparently normal calves, whereas of the 8 controls 7 aborted.

Of the seven animals which received strain 11, six produced vigorous calves. *Brucella abortus* was isolated from the uterus and colostrum of one of the animals (No. 1533) and from the uterus of another (No. 1536), which died while giving birth to a calf. *Brucella abortus* was not isolated from the uterus or colostrum of the other five animals of this group at the time of parturition.

TABLE 4.—Immunizing results with cows and heifers injected with vaccine prepared from *Brucella abortus* strains of different degrees of virulence, the animals later being subjected to artificial exposure to *Brucella abortus* infected materials

GROUP VACCINATED WITH STRAIN 11 VACCINE (OF LOW VIRULENCE)

Animal No.	Date of vaccination, 1929	Dose	Date of breeding	Date of exposure	Gestation period	Outcome of pregnancy	Results of uterine examination for <i>Br. abortus</i>	Results of colostrum examination for <i>Br. abortus</i>
Cow:		C c			Days			
1462..	Apr. 23	20	{Aug. 31, 1929 Dec. 23, 1929	Feb. 24, 1930	277	Vigorous calf...	Negative..	Negative.
1498..	do.....	.25	July 11, 1929	Oct. 19, 1929	274	do.....	do.....	Do.
Heifer:								
1441..	do.....	20	{Aug. 1, 1929 Aug. 17, 1929	do.....	282	do.....	do.....	Do.
1492..	do.....	10	{Jan. 9, 1930 Mar. 2, 1930	Feb. 24, 1930	278	do.....	do.....	Do.
1493..	do.....	20	Aug. 8, 1929	June 10, 1930	278	do.....	do.....	Do.
1533..	do.....	20	(a)	Oct. 19, 1929	265	do.....	Positive..	Positive.
1536..	do.....	10		do.....		Dam died during calving.	do.....	

GROUP VACCINATED WITH STRAIN 19 VACCINE (OF MEDIUM VIRULENCE)

Cow:								
1452..	Apr. 23	20	{June 20, 1929 June 25, 1929	Oct. 19, 1929	279	Vigorous calf...	Negative..	Negative.
1490..	do.....	.25	{July 23, 1929 June 12, 1929	do.....	281	do.....	Positive..	Positive.
1501..	do.....	10	do.....	do.....	272	do.....	do.....	Negative.
Heifer:								
1451..	do.....	20	July 27, 1929	do.....	275	do.....	do.....	Do.
1495..	do.....	10	June 17, 1929	do.....	278	do.....	Negative..	Do.
1505..	do.....	20	Nov. 1, 1929	Feb. 24, 1930	274	do.....	do.....	Do.
1529..	do.....	10	{Jan. 29, 1930 Apr. 12, 1930	June 10, 1930	278	do.....	do.....	Do.
1537..	do.....	10	(a)	Oct. 19, 1929		do.....	do.....	Do.

GROUP VACCINATED WITH STRAIN 484 VACCINE (OF HIGH VIRULENCE)

Cow:								
1502..	Apr. 23	0.25	{June 14, 1929 July 9, 1929	Oct. 19, 1929	288	Vigorous calf...	Negative..	Positive.
1504..	do.....	10	{June 13, 1929 July 11, 1929 Oct. 15, 1929 Nov. 6, 1929	Feb. 24, 1930	264	Weak calf.....	Positive..	Do.
Heifer:								
1386..	do.....	20	(a)	Oct. 22, 1930		Vigorous calf...	Negative..	Negative.
1396..	do.....	10	(a)	Oct. 19, 1929		do.....	do.....	Do.
1491..	do.....	20	{July 20, 1929 Aug. 10, 1929 Oct. 31, 1929 Dec. 7, 1929 Jan. 10, 1930 Feb. 8, 1930	June 10, 1930	271	do.....	do.....	Do.
1499..	do.....	10	June 21, 1929	Oct. 19, 1929	280	do.....	do.....	Do.
1528..	do.....	20	Feb. 10, 1930	June 10, 1930	283	do.....	do.....	Do.
1532..	do.....	10	Mar. 18, 1930	do.....	272	do.....	do.....	Do.

CONTROLS, NOT VACCINATED

Heifer:								
1427..			May 27, 1929	Oct. 19, 1929	275	Vigorous calf...	Positive..	Positive.
1429..			June 6, 1929	do.....	218	Abortion.....	do.....	Do.
1447..			{May 8, 1929 June 21, 1929	do.....	158	do.....	do.....	Do.
1489..			June 21, 1929	do.....	177	do.....	do.....	Do.
1548..			(a)	do.....		do.....	do.....	Do.
1446..			Sept. 3, 1929	Feb. 24, 1930	195	do.....	do.....	Do.
1625..			Apr. 2, 1930	June 10, 1930	157	do.....	do.....	Do.
1584..			{Dec. 5, 1929 Apr. 3, 1930 July 16, 1930	Oct. 22, 1930	181	do.....	do.....	Do.

\* No record.



VACCINATED COW 1498

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	—	—	—	—	—	—	Apr. 23, 1929, intradermic injection of $\frac{1}{4}$ c c abortion vaccine ( <i>Br. abortus</i> strain 11).
Apr. 23, 1929	—	—	—	—	—	—	Apr. 30, 1929, milk negative for <i>Br. abortus</i> .
Apr. 30, 1929	+	+	+	P	—	—	May 7, 1929, milk negative for <i>Br. abortus</i> .
May 7, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for <i>Br. abortus</i> .
May 14, 1929	+	+	+	+	+	+	May 21, 1929, milk positive for <i>Br. abortus</i> .
May 21, 1929	+	+	+	+	+	P	May 28, 1929, milk negative for <i>Br. abortus</i> .
June 4, 1929	+	+	+	+	+	P	June 4, 1929, milk negative for <i>Br. abortus</i> .
June 18, 1929	+	+	P	P	P	P	June 11, 1929, bred.
July 2, 1929	+	+	+	+	+	+	Aug. 13, 1929, milk negative for <i>Br. abortus</i> .
July 17, 1929	+	+	+	+	+	—	Sept. 21, 1929, milk negative for <i>Br. abortus</i> .
July 31, 1929	+	+	+	P	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Aug. 27, 1929	+	+	+	+	S	—	Oct. 21, 1929, milk negative for <i>Br. abortus</i> .
Sept. 11, 1929	+	+	+	+	—	—	
Sept. 24, 1929	+	+	+	+	—	—	
Oct. 8, 1929	+	+	P	P	—	—	
Oct. 21, 1929	+	+	+	P	S	—	
Nov. 6, 1929	+	+	+	P	—	—	
Nov. 19, 1929	+	+	P	+	—	—	
Dec. 19, 1929	+	+	+	+	—	—	
Jan. 8, 1930	+	+	+	+	—	—	
Jan. 22, 1930	+	+	+	+	—	—	
Feb. 5, 1930	+	+	+	+	—	—	
Feb. 19, 1930	+	+	+	+	—	—	
Mar. 5, 1930	+	+	+	+	—	—	
Mar. 19, 1930	+	+	P	—	—	—	
Apr. 2, 1930	+	+	+	P	—	—	Apr. 11, 1930, produced a vigorous calf (274 days); guinea pigs inoculated with uterine material and colostrum did not acquire abortion disease.
Apr. 16, 1930	+	+	+	P	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
July 16, 1930	+	+	+	+	—	—	
Aug. 19, 1930	+	+	+	P	—	—	

## VACCINATED HEIFER 1441

Apr. 10, 1929	-	-	-	-	-	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 11).
Apr. 23, 1929	-	-	-	-	-	
Apr. 30, 1929	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	
June 18, 1929	+	+	+	+	+	
July 2, 1929	+	+	+	S	-	
July 17, 1929	+	+	+	+	-	
July 31, 1929	+	+	P	-	-	
Aug. 27, 1929	+	+	P	-	-	Aug. 1, 1929, bred.
Sept. 11, 1929	+	+	-	-	-	
Sept. 24, 1929	+	+	+	+	-	
Oct. 8, 1929	+	+	+	S	-	
Oct. 21, 1929	+	+	-	-	-	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929	+	+	+	-	-	
Nov. 19, 1929	+	+	-	-	-	
Dec. 19, 1929	+	+	S	-	-	
Jan. 8, 1930	+	+	-	-	-	
Jan. 22, 1930	+	+	+	-	-	
Feb. 5, 1930	+	P	-	-	-	
Feb. 19, 1930	+	P	-	-	-	
Mar. 5, 1930	+	+	-	-	-	
Mar. 19, 1930	+	P	-	-	-	
Apr. 2, 1930	+	+	S	-	-	
Apr. 16, 1930	+	+	-	-	-	May 10, 1930, produced a vigorous calf (282 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Apr. 22, 1930	+	P	-	-	-	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
July 16, 1930	+	P	-	-	-	
Aug. 19, 1930	+	+	P	-	-	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1492

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 11).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	+	+	
June 13, 1929.....	+	+	+	+	—	P	
July 2, 1929.....	+	+	+	+	—	—	
July 17, 1929.....	+	+	+	+	—	—	
July 31, 1929.....	+	+	+	—	—	—	Aug. 17, 1929, bred.
Aug. 27, 1929.....	+	+	S	S	—	—	
Sept. 11, 1929.....	+	+	+	—	—	—	
Sept. 24, 1929.....	+	+	+	—	—	—	
Oct. 8, 1929.....	+	+	+	—	—	—	
Oct. 21, 1929.....	+	+	+	—	—	—	
Nov. 6, 1929.....	+	+	P	—	—	—	
Nov. 19, 1929.....	+	+	P	—	—	—	
Dec. 19, 1929.....	+	+	+	S	—	—	
Jan. 8, 1930.....	+	+	P	—	—	—	Jan. 9, 1930, bred. Feb. 24, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Feb. 5, 1930.....	+	+	+	—	—	—	
Mar. 5, 1930.....	+	+	+	+	—	—	
Mar. 19, 1930.....	+	+	+	S	—	—	
Apr. 2, 1930.....	+	+	+	S	—	—	
Apr. 16, 1930.....	+	+	+	—	—	—	
Apr. 22, 1930.....	+	+	P	—	—	—	
May 7, 1930.....	+	+	+	—	—	—	
May 17, 1930.....	+	+	+	—	—	—	
June 28, 1930.....	+	+	+	S	—	—	
July 16, 1930.....	+	+	P	P	—	—	Oct. 14, 1930, produced a vigorous calf (278 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Aug. 19, 1930.....	+	+	+	P	—	—	
Jan. 9, 1931.....	+	+	+	P	—	—	

## VACCINATED HEIFER 1493

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 11).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	P	
June 4, 1929.....	+	+	+	+	+	—	
June 18, 1929.....	+	+	+	+	+	—	
July 2, 1929.....	+	+	+	+	—	—	
July 17, 1929.....	+	+	+	P	—	—	
July 31, 1929.....	+	+	+	—	—	—	Mar. 26, 1930, bred.
Aug. 27, 1929.....	+	P	S	—	—	—	
Sept. 11, 1929.....	P	P	—	—	—	—	
Sept. 24, 1929.....	+	+	P	—	—	—	
Oct. 8, 1929.....	+	+	—	—	—	—	
Oct. 21, 1929.....	+	+	—	—	—	—	
Nov. 6, 1929.....	P	—	—	—	—	—	
Nov. 19, 1929.....	+	—	—	—	—	—	
Dec. 19, 1929.....	+	S	—	—	—	—	
Jan. 8, 1930.....	—	—	—	—	—	—	June 10, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Feb. 5, 1930.....	+	S	—	—	—	—	
Mar. 5, 1930.....	+	S	—	—	—	—	
Apr. 2, 1930.....	P	S	—	—	—	—	
May 17, 1930.....	+	+	P	—	—	—	
June 17, 1930.....	+	+	+	+	—	—	
June 28, 1930.....	+	+	+	P	—	—	
July 16, 1930.....	+	+	+	S	—	—	
Aug. 19, 1930.....	+	+	+	—	—	—	
Oct. 2, 1930.....	+	+	+	—	—	—	Dec. 29, 1930, produced a vigorous calf (278 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Nov. 11, 1930.....	+	+	P	—	—	—	
Nov. 22, 1930.....	P	P	—	—	—	—	
Jan. 9, 1931.....	+	+	+	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1533

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 11).
Apr. 23, 1929....	—	—	—	—	—	—	
Apr. 30, 1929....	+	+	+	+	+	+	
May 7, 1929....	+	+	+	+	+	+	
May 14, 1929....	+	+	+	+	P	—	
May 21, 1929....	+	+	+	+	P	—	Aug. 8, 1929, bred.
June 4, 1929....	+	+	+	+	—	—	
June 18, 1929....	+	+	+	+	—	—	
July 2, 1929....	+	+	+	—	—	—	
July 17, 1929....	+	+	P	—	—	—	
July 31, 1929....	P	—	—	—	—	—	
Aug. 27, 1929....	P	S	—	—	—	—	
Sept. 11, 1929....	—	—	—	—	—	—	
Sept. 24, 1929....	P	—	—	—	—	—	
Oct. 8, 1929....	P	—	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Oct. 21, 1929....	+	—	—	—	—	—	
Nov. 6, 1929....	+	+	—	—	—	—	
Nov. 19, 1929....	+	+	P	—	—	—	
Dec. 19, 1929....	S	—	—	—	—	—	
Jan. 8, 1930....	+	—	—	—	—	—	
Jan. 22, 1930....	+	+	—	—	—	—	
Feb. 5, 1930....	+	—	—	—	—	—	
Feb. 19, 1930....	S	—	—	—	—	—	
Mar. 5, 1930....	—	—	—	—	—	—	
Mar. 19, 1930....	—	—	—	—	—	—	Apr. 30, 1930, produced a vigorous calf (265 days); 4 out of 6 guinea pigs inoculated with uterine material and 1 pig out of 6 inoculated with colostrum acquired abortion disease.
Apr. 2, 1930....	—	—	—	—	—	—	
Apr. 16, 1930....	—	—	—	—	—	—	
Apr. 22, 1930....	—	—	—	—	—	—	
July 16, 1930....	—	—	—	—	—	—	
Aug. 19, 1930....	—	—	—	—	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .

## VACCINATED HEIFER 1536

Apr. 10, 1929....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 11).
Apr. 23, 1929....	+	+	+	+	+	+	
Apr. 30, 1929....	+	+	+	+	+	+	
May 7, 1929....	+	+	+	+	+	+	
May 14, 1929....	+	+	+	+	+	+	
May 21, 1929....	+	+	+	+	+	S	No breeding record.
June 4, 1929....	+	+	+	+	—	—	
June 18, 1929....	+	+	+	+	—	—	
July 2, 1929....	+	+	+	+	—	—	
July 17, 1929....	+	+	+	—	—	—	
July 31, 1929....	+	+	+	—	—	—	
Aug. 27, 1929....	+	P	S	—	—	—	
Sept. 11, 1929....	+	+	—	—	—	—	
Sept. 24, 1929....	+	+	—	—	—	—	
Oct. 8, 1929....	+	+	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure. Nov. 29, 1929, dam died while calving; guinea pigs inoculated with uterine material acquired abortion disease. (Dam seemingly vaccinated by mistake when bred.)
Oct. 21, 1929....	+	+	—	—	—	—	
Nov. 6, 1929....	+	+	+	—	—	—	
Nov. 19, 1929....	+	+	—	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED COW 1452

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 30, 1929.....	+	+	+	+	+	+	May 7, 1929, milk negative for <i>Br. abortus</i> .
May 7, 1929.....	+	+	+	+	+	+	May 14, 1929, milk negative for <i>Br. abortus</i> .
May 14, 1929.....	+	+	+	+	+	+	May 28, 1929, milk negative for <i>Br. abortus</i> .
May 21, 1929.....	+	+	+	+	+	+	June 11, 1929, milk negative for <i>Br. abortus</i> .
June 4, 1929.....	+	+	+	+	+	—	June 18, 1929, milk negative for <i>Br. abortus</i> .
June 18, 1929.....	+	+	+	+	—	—	June 20, 1929, bred.
July 2, 1929.....	+	+	+	P	—	—	July 2, 1929, milk negative for <i>Br. abortus</i> .
July 17, 1929.....	+	+	+	P	—	—	
July 31, 1929.....	+	+	+	—	—	—	Aug. 13, 1929, milk negative for <i>Br. abortus</i> .
Aug. 27, 1929.....	+	+	—	—	—	—	Sept. 21, 1929, milk negative for <i>Br. abortus</i> .
Sept. 11, 1929.....	+	+	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Sept. 24, 1929.....	+	P	P	—	—	—	Oct. 21, 1929, milk negative for <i>Br. abortus</i> .
Oct. 8, 1929.....	+	+	P	—	—	—	
Oct. 21, 1929.....	+	+	—	—	—	—	
Nov. 6, 1929.....	+	+	+	—	—	—	
Nov. 19, 1929.....	+	+	+	+	—	—	
Dec. 19, 1929.....	+	+	+	—	—	—	
Jan. 8, 1930.....	+	+	—	—	—	—	
Jan. 22, 1930.....	+	+	+	—	—	—	
Feb. 5, 1930.....	+	+	S	—	—	—	
Feb. 19, 1930.....	+	+	P	—	—	—	
Mar. 5, 1930.....	+	+	—	—	—	—	
Mar. 19, 1930.....	+	P	—	—	—	—	Mar. 26, 1930, produced a vigorous calf (279 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Apr. 2, 1930.....	+	+	P	—	—	—	
Apr. 16, 1930.....	+	P	—	—	—	—	
July 16, 1930.....	+	+	—	—	—	—	

## VACCINATED COW 1490

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, intradermic injection of ¼ c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 30, 1929, milk positive for <i>Br. abortus</i> .
Apr. 30, 1929.....	+	—	P	P	—	—	May 7, 1929, milk positive for <i>Br. abortus</i> .
May 7, 1929.....	+	+	+	+	+	P	May 14, 1929, milk positive for <i>Br. abortus</i> .
May 14, 1929.....	+	+	+	+	+	+	May 21, 1929, milk positive for <i>Br. abortus</i> .
May 21, 1929.....	+	+	+	+	+	+	May 28, 1929, milk positive for <i>Br. abortus</i> .
June 4, 1929.....	+	+	+	+	+	+	June 4, 1929, milk positive for <i>Br. abortus</i> .
June 18, 1929.....	+	+	+	+	+	+	June 11, 1929, milk positive for <i>Br. abortus</i> .
July 2, 1929.....	+	+	+	+	+	+	June 18, 1929, milk positive for <i>Br. abortus</i> .
July 17, 1929.....	+	+	+	+	+	+	June 25, 1929, bred.
July 31, 1929.....	+	+	+	+	+	+	July 2, 1929, milk positive for <i>Br. abortus</i> .
Aug. 27, 1929.....	+	+	+	+	+	+	July 23, 1929, bred.
Sept. 11, 1929.....	+	+	+	+	+	P	Aug. 13, 1929, milk positive for <i>Br. abortus</i> .
Sept. 24, 1929.....	+	+	+	+	+	+	Sept. 21, 1929, milk positive for <i>Br. abortus</i> .
Oct. 8, 1929.....	+	+	+	+	+	+	Oct. 21, 1929, milk positive for <i>Br. abortus</i> .
Oct. 21, 1929.....	+	+	+	+	+	+	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929.....	+	+	+	+	+	+	
Nov. 19, 1929.....	+	+	+	+	+	+	
Dec. 19, 1929.....	+	+	+	+	+	+	
Jan. 8, 1930.....	+	+	+	+	+	+	
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Feb. 19, 1930.....	+	+	+	+	+	+	
Mar. 5, 1930.....	+	+	+	+	+	P	
Mar. 19, 1930.....	+	+	+	+	+	P	
Apr. 2, 1930.....	+	+	+	+	+	+	Apr. 30, 1930, produced a vigorous calf (281 days); guinea pigs inoculated with uterine material and colostrum acquired abortion disease.
Apr. 16, 1930.....	+	+	+	+	+	+	
Apr. 22, 1930.....	+	+	+	+	+	—	
July 16, 1930.....	+	+	+	+	+	+	
Aug. 19, 1930.....	+	+	+	+	+	+	Aug. 16, 1930, milk positive for <i>Br. abortus</i> .
Nov. 11, 1930.....	+	+	+	+	+	+	Aug. 22, 1930, milk positive for <i>Br. abortus</i> .
Nov. 22, 1930.....	+	+	+	+	+	+	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED COW 1501

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929	—	—	—	—	—	—	
Apr. 23, 1929	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 30, 1929	+	+	+	+	+	+	Apr. 30, 1929, milk negative for <i>Br. abortus</i> .
May 7, 1929	+	+	+	+	+	+	May 7, 1929, milk negative for <i>Br. abortus</i> .
May 14, 1929	+	+	+	+	+	+	May 14, 1929, milk negative for <i>Br. abortus</i> .
May 21, 1929	+	+	+	+	+	+	May 21, 1929, milk negative for <i>Br. abortus</i> .
June 4, 1929	+	+	+	+	+	+	May 28, 1929, milk negative for <i>Br. abortus</i> .
June 18, 1929	+	+	+	+	+	—	June 4, 1929, milk negative for <i>Br. abortus</i> .
July 2, 1929	+	+	+	+	+	—	June 11, 1929, milk negative for <i>Br. abortus</i> .
July 17, 1929	+	+	+	+	+	—	June 12, 1929, bred.
July 31, 1929	+	+	+	+	—	—	June 18, 1929, milk negative for <i>Br. abortus</i> .
Aug. 27, 1929	+	+	+	—	—	—	July 2, 1929, milk negative for <i>Br. abortus</i> .
Sept. 11, 1929	+	+	+	—	—	—	Aug. 13, 1929, milk negative for <i>Br. abortus</i> .
Sept. 24, 1929	+	+	+	—	—	—	Sept. 21, 1929, milk negative for <i>Br. abortus</i> .
Oct. 8, 1929	+	+	+	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Oct. 21, 1929	+	+	+	—	—	—	Oct. 21, 1929, milk negative for <i>Br. abortus</i> .
Nov. 6, 1929	+	+	+	+	—	—	
Nov. 19, 1929	+	+	+	+	—	—	
Dec. 19, 1929	+	+	+	+	—	—	
Jan. 8, 1930	+	+	+	P	—	—	
Jan. 22, 1930	+	+	+	+	—	—	
Feb. 5, 1930	+	+	+	+	—	—	
Feb. 19, 1930	+	+	+	P	—	—	
Mar. 5, 1930	+	+	+	—	—	—	Mar. 11, 1930, produced a vigorous calf (272 days); guinea pigs inoculated with uterine material acquired abortion disease, whereas colostrum failed to infect them.
Mar. 19, 1930	+	+	+	—	—	—	
Apr. 2, 1930	+	+	+	—	—	—	
Apr. 16, 1930	+	+	+	P	—	—	
July 16, 1930	+	+	+	S	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Aug. 19, 1930	+	+	+	—	—	—	
Oct. 2, 1930	+	P	+	—	—	—	
Nov. 11, 1930	+	+	S	—	—	—	
Nov. 22, 1930	+	+	+	—	—	—	
Jan. 9, 1931	+	+	+	—	—	—	Jan. 24, 1931, milk negative for <i>Br. abortus</i> .

## VACCINATED HEIFER 1451

Apr. 10, 1929	—	—	—	—	—	—	
Apr. 23, 1929	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 30, 1929	+	+	+	+	+	+	
May 7, 1929	+	+	+	+	+	+	
May 14, 1929	+	+	+	+	+	+	
May 21, 1929	+	+	+	+	+	+	
June 4, 1929	+	+	+	+	+	—	
June 18, 1929	+	+	+	+	+	—	
July 2, 1929	+	+	+	+	+	—	
July 17, 1929	+	+	+	+	P	—	
July 31, 1929	+	+	+	+	—	—	July 27, 1929, bred.
Aug. 27, 1929	+	+	+	—	—	—	
Sept. 11, 1929	+	+	+	—	—	—	
Sept. 24, 1929	+	+	+	—	—	—	
Oct. 8, 1929	+	+	+	—	—	—	
Oct. 21, 1929	+	+	S	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929	+	+	+	+	—	—	
Nov. 19, 1929	+	+	+	+	—	—	
Dec. 19, 1929	+	+	+	+	—	—	
Jan. 8, 1930	+	+	+	+	—	—	
Jan. 22, 1930	+	+	+	+	—	—	
Feb. 5, 1930	+	+	+	P	—	—	
Feb. 19, 1930	+	+	+	+	P	—	
Mar. 5, 1930	+	+	+	P	—	—	
Mar. 19, 1930	+	+	+	—	—	—	
Apr. 2, 1930	+	+	—	—	—	—	
Apr. 16, 1930	+	S	—	—	—	—	Apr. 28, 1930, produced a vigorous calf (275 days); 2 out of 6 guinea pigs inoculated with uterine material acquired abortion disease; colostrum failed to infect guinea pigs.
Apr. 22, 1930	+	+	P	—	—	—	
July 16, 1930	+	+	+	P	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Aug. 19, 1930	+	+	+	—	—	—	



TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1495

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	+	—	June 17, 1929, bred.
June 18, 1929.....	+	+	+	+	—	—	
July 2, 1929.....	+	+	+	+	—	—	
July 17, 1929.....	+	+	+	+	—	—	
July 31, 1929.....	+	+	+	S	—	—	
Aug. 27, 1929.....	+	+	+	S	—	—	
Sept. 11, 1929.....	+	P	+	S	—	—	
Sept. 24, 1929.....	+	+	+	—	—	—	
Oct. 8, 1929.....	+	+	+	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Oct. 21, 1929.....	+	+	+	—	—	—	
Nov. 6, 1929.....	+	+	+	+	—	—	
Nov. 19, 1929.....	+	+	+	P	—	—	
Dec. 19, 1929.....	+	+	+	—	—	—	
Jan. 8, 1930.....	+	+	+	—	—	—	
Jan. 22, 1930.....	+	+	P	—	—	—	
Feb. 5, 1930.....	+	+	+	—	—	—	
Feb. 19, 1930.....	+	+	+	—	—	—	
Mar. 5, 1930.....	+	+	P	—	—	—	
Mar. 19, 1930.....	+	+	P	—	—	—	Mar. 22, 1930, produced a vigorous calf (278 days); guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
Apr. 2, 1930.....	+	+	—	—	—	—	
Apr. 16, 1930.....	+	+	S	—	—	—	
July 16, 1930.....	+	+	—	S	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Aug. 19, 1930.....	+	+	P	—	—	—	Oct. 4, 1930, milk negative for <i>Br. abortus</i> .

## VACCINATED HEIFER 1505

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	—	—	
June 18, 1929.....	+	+	+	+	—	—	
July 2, 1929.....	+	+	+	+	—	—	
July 17, 1929.....	+	+	+	+	—	—	
July 31, 1929.....	+	+	+	—	—	—	
Aug. 27, 1929.....	+	+	S	—	—	—	
Sept. 11, 1929.....	+	+	+	P	—	—	
Sept. 24, 1929.....	+	+	+	—	—	—	
Oct. 8, 1929.....	+	+	P	S	—	—	
Oct. 21, 1929.....	+	+	+	—	—	—	Nov. 1, 1929, bred.
Nov. 6, 1929.....	+	P	—	—	—	—	
Nov. 19, 1929.....	+	+	P	—	—	—	
Dec. 19, 1929.....	+	+	P	—	—	—	
Jan. 8, 1930.....	+	P	S	—	—	—	
Jan. 22, 1930.....	+	+	P	—	—	—	
Feb. 5, 1930.....	+	+	P	—	—	—	Feb. 24, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Mar. 5, 1930.....	+	+	—	—	—	—	
Mar. 19, 1930.....	+	+	P	—	—	—	
Apr. 2, 1930.....	+	+	+	—	—	—	Aug. 2, 1930, produced a vigorous calf (274 days); guinea pigs inoculated with uterine material and colostrum did not acquire abortion disease.
Apr. 16, 1930.....	+	+	—	—	—	—	
Apr. 22, 1930.....	+	+	—	—	—	—	
May 7, 1930.....	+	+	—	—	—	—	
May 17, 1930.....	+	+	+	—	—	—	
June 17, 1930.....	+	+	—	S	—	—	
June 28, 1930.....	+	+	+	S	—	—	
July 16, 1930.....	+	+	—	S	—	—	
Aug. 19, 1930.....	+	+	P	—	—	—	
Oct. 2, 1930.....	+	P	—	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1529

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	+	P	
June 18, 1929.....	+	+	+	+	—	—	
July 2, 1929.....	+	+	+	+	+	—	
July 17, 1929.....	+	+	P	P	—	—	
July 31, 1929.....	+	+	—	—	—	—	
Aug. 27, 1929.....	+	P	S	—	—	—	
Sept. 11, 1929.....	+	—	P	—	—	—	
Sept. 24, 1929.....	+	—	P	—	—	—	
Oct. 8, 1929.....	+	P	S	—	—	—	
Oct. 21, 1929.....	+	P	P	—	—	—	
Nov. 6, 1929.....	+	—	—	—	—	—	
Nov. 19, 1929.....	+	+	—	—	—	—	
Dec. 19, 1929.....	+	+	P	—	—	—	
Jan. 8, 1930.....	+	+	—	—	—	—	Jan. 29, 1930, bred.
Feb. 5, 1930.....	+	+	S	—	—	—	
Mar. 5, 1930.....	+	+	S	—	—	—	Apr. 12, 1930, bred.
Apr. 2, 1930.....	+	+	—	—	—	—	
June 11, 1930.....	+	+	+	+	—	—	June 10, 1930, received <i>Br. abortus</i> , conjunctival exposure.
June 17, 1930.....	+	+	—	S	—	—	
June 28, 1930.....	+	+	+	+	S	—	Jan. 15, 1931, produced a vigorous calf (278 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
July 16, 1930.....	+	+	+	+	—	—	
Aug. 19, 1930.....	+	+	+	+	—	—	Mar. 20, 1931, milk negative for <i>Br. abortus</i> .
Oct. 2, 1930.....	+	+	+	+	—	—	
Nov. 11, 1930.....	+	+	S	—	—	—	

## VACCINATED HEIFER 1537

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 19).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	No breeding record. (Was vaccinated by mistake when about one month pregnant.)
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	+	—	
June 18, 1929.....	+	+	+	+	+	—	
July 2, 1929.....	+	+	+	—	—	—	
July 17, 1929.....	+	+	+	—	—	—	
July 31, 1929.....	+	—	S	—	—	—	
Aug. 27, 1929.....	+	P	—	—	—	—	
Sept. 11, 1929.....	+	—	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Sept. 24, 1929.....	+	—	—	—	—	—	
Oct. 8, 1929.....	+	—	—	—	—	—	Dec. 21, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Oct. 21, 1929.....	P	—	—	—	—	—	
Nov. 6, 1929.....	+	+	—	—	—	—	
Nov. 19, 1929.....	+	+	—	—	—	—	
Dec. 19, 1929.....	+	P	—	—	—	—	
Jan. 8, 1930.....	—	—	—	—	—	—	
Jan. 22, 1930.....	+	+	—	—	—	—	
Feb. 5, 1930.....	P	—	—	—	—	—	
Feb. 19, 1930.....	P	—	—	—	—	—	
Mar. 5, 1930.....	—	—	—	—	—	—	
Mar. 19, 1930.....	—	—	—	—	—	—	
Apr. 2, 1930.....	—	—	—	—	—	—	
Apr. 16, 1930.....	—	—	—	—	—	—	
July 16, 1930.....	+	P	S	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED COW 1502

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, intradermic injection of $\frac{1}{4}$ c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	P	—	—	—	Apr. 30, 1929, milk negative for <i>Br. abortus</i> .
May 2, 1929.....	+	+	P	—	—	—	May 7, 1929, milk negative for <i>Br. abortus</i> .
May 7, 1929.....	+	+	+	+	+	—	May 14, 1929, milk negative for <i>Br. abortus</i> .
May 14, 1929.....	+	+	+	+	P	—	May 21, 1929, milk positive for <i>Br. abortus</i> .
May 21, 1929.....	+	+	+	+	+	—	May 28, 1929, milk positive for <i>Br. abortus</i> .
June 4, 1929.....	+	+	+	+	+	—	June 4, 1929, milk positive for <i>Br. abortus</i> .
June 18, 1929.....	+	+	+	+	+	P	June 11, 1929, milk positive for <i>Br. abortus</i> .
July 2, 1929.....	+	+	+	+	+	P	June 14, 1929, bred.
July 17, 1929.....	+	+	+	+	+	+	June 18, 1929, milk positive for <i>Br. abortus</i> .
July 31, 1929.....	+	+	+	+	+	+	July 2, 1929, milk positive for <i>Br. abortus</i> .
Aug. 27, 1929.....	+	+	+	+	+	S	July 9, 1929, bred.
Sept. 11, 1929.....	+	+	+	+	+	+	Aug. 13, 1929, milk positive for <i>Br. abortus</i> .
Sept. 24, 1929.....	+	+	+	+	+	+	Sept. 21, 1929, milk positive for <i>Br. abortus</i> .
Oct. 8, 1929.....	+	+	+	+	+	+	Oct. 21, 1929, milk positive for <i>Br. abortus</i> .
Oct. 21, 1929.....	+	+	+	+	+	+	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929.....	+	+	+	+	+	+	
Nov. 19, 1929.....	+	+	+	+	+	+	
Dec. 19, 1929.....	+	+	+	+	+	+	
Jan. 8, 1930.....	+	+	+	+	+	+	
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Mar. 5, 1930.....	+	+	+	+	+	+	
Mar. 19, 1930.....	+	+	+	+	+	+	
Apr. 2, 1930.....	+	+	+	+	+	+	Apr. 23, 1930, produced a vigorous calf (288 days); guinea pigs inoculated with uterine material failed to acquire abortion disease; guinea pigs were infected with abortion disease by colostrum injections.
Apr. 16, 1930.....	+	+	+	+	+	+	Aug. 16, 1930, milk positive for <i>Br. abortus</i> .
Apr. 22, 1930.....	+	+	+	+	+	+	Aug. 22, 1930, milk positive for <i>Br. abortus</i> .
July 16, 1930.....	+	+	+	+	+	+	Jan. 26, 1931, milk positive for <i>Br. abortus</i> .
Aug. 19, 1930.....	+	+	+	+	+	+	
Nov. 11, 1930.....	+	+	+	+	+	+	
Nov. 22, 1930.....	+	+	+	+	+	+	
Jan. 9, 1931.....	+	+	+	+	+	+	

## VACCINATED COW 1504

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	—	—	Apr. 30, 1929, milk negative for <i>Br. abortus</i> .
May 2, 1929.....	+	+	+	+	+	—	May 7, 1929, milk positive for <i>Br. abortus</i> .
May 7, 1929.....	+	+	+	+	+	+	May 14, 1929, milk positive for <i>Br. abortus</i> .
May 14, 1929.....	+	+	+	+	+	+	May 21, 1929, milk positive for <i>Br. abortus</i> .
May 21, 1929.....	+	+	+	+	+	+	May 28, 1929, milk positive for <i>Br. abortus</i> .
June 4, 1929.....	+	+	+	+	+	+	June 4, 1929, milk positive for <i>Br. abortus</i> .
June 18, 1929.....	+	+	+	+	+	+	June 13, 1929, bred.
July 2, 1929.....	+	+	+	+	+	+	June 11, 1929, milk positive for <i>Br. abortus</i> .
July 17, 1929.....	+	+	+	+	+	+	June 18, 1929, milk positive for <i>Br. abortus</i> .
July 31, 1929.....	+	+	+	+	+	+	July 2, 1929, milk positive for <i>Br. abortus</i> .
Aug. 27, 1929.....	+	+	+	+	+	+	July 11, 1929, bred.
Sept. 11, 1929.....	+	+	+	+	+	+	Aug. 13, 1929, milk positive for <i>Br. abortus</i> .
Sept. 24, 1929.....	+	+	+	+	+	+	Sept. 21, 1929, milk positive for <i>Br. abortus</i> .
Oct. 8, 1929.....	+	+	+	+	+	+	Oct. 15, 1929, bred.
Oct. 21, 1929.....	+	+	+	+	+	+	Oct. 21, 1929, milk positive for <i>Br. abortus</i> .
Nov. 6, 1929.....	+	+	+	+	+	+	Nov. 6, 1929, bred.
Nov. 19, 1929.....	+	+	+	+	+	+	
Dec. 19, 1929.....	+	+	+	+	+	+	
Jan. 8, 1930.....	+	+	+	+	+	+	Feb. 24, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Mar. 5, 1930.....	+	+	+	+	+	+	
Apr. 2, 1930.....	+	+	+	+	+	+	
Apr. 16, 1930.....	+	+	+	+	+	+	July 28, 1930, produced a weak calf (264 days); guinea pigs which were inoculated both with uterine material and colostrum became affected with abortion disease.
Apr. 22, 1930.....	+	+	+	+	+	+	
May 7, 1930.....	+	+	+	+	+	+	
May 17, 1930.....	+	+	+	+	+	+	
June 17, 1930.....	+	+	+	+	+	+	
June 28, 1930.....	+	+	+	+	+	+	
July 16, 1930.....	+	+	+	+	+	+	
Aug. 19, 1930.....	+	+	+	+	+	+	Aug. 16, 1930, milk positive for <i>Br. abortus</i> .
Nov. 11, 1930.....	+	+	+	+	+	+	Aug. 22, 1930, milk positive for <i>Br. abortus</i> .
Nov. 22, 1930.....	+	+	+	+	+	+	
Jan. 9, 1931.....	+	+	+	+	+	+	Jan. 24, 1931, milk positive for <i>Br. abortus</i> .

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1386

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 30, 1929.....	+	+	+	+	+	P	
May 2, 1929.....	+	+	+	+	+	P	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	P	—	No breeding record.
June 18, 1929.....	+	+	+	+	+	P	
July 2, 1929.....	+	+	+	+	+	+	
July 17, 1929.....	+	+	+	+	+	—	
July 31, 1929.....	+	+	+	+	—	—	
Aug. 27, 1929.....	+	+	P	S	—	—	
Sept. 11, 1929.....	+	+	+	+	—	—	
Sept. 24, 1929.....	+	+	+	+	—	—	
Oct. 8, 1929.....	+	+	+	+	—	—	
Oct. 21, 1929.....	+	+	P	—	—	—	
Nov. 6, 1929.....	+	+	+	—	—	—	
Nov. 19, 1929.....	+	+	+	—	—	—	
Dec. 19, 1929.....	+	S	—	—	—	—	
Jan. 8, 1930.....	+	P	—	—	—	—	
Feb. 5, 1930.....	P	P	—	—	—	—	
Mar. 5, 1930.....	+	+	P	—	—	—	Mar. 3, 1931, produced a vigorous calf; guinea pigs inoculated with uterine material and colostrum did not acquire abortion disease.
Apr. 2, 1930.....	+	+	+	—	—	—	
Apr. 16, 1930.....	+	+	+	—	—	—	
July 16, 1930.....	+	+	+	+	—	—	
Aug. 19, 1930.....	+	+	+	+	—	—	
Sept. 30, 1930.....	+	+	+	P	—	—	
Oct. 22, 1930.....	+	+	P	S	—	—	Oct. 22, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 22, 1930.....	+	+	+	+	+	—	
Feb. 19, 1931.....	+	+	+	—	—	—	
June 2, 1931.....	+	+	+	—	—	—	

## VACCINATED HEIFER 1396

Apr. 10, 1929.....	—	—	—	—	—	—	
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
May 2, 1929.....	+	+	+	+	+	P	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	+	+	No breeding record.
June 18, 1929.....	+	+	+	+	+	+	
July 2, 1929.....	+	+	+	+	+	+	
July 17, 1929.....	+	+	+	+	+	—	
July 31, 1929.....	+	+	+	+	+	—	
Aug. 27, 1929.....	+	+	+	S	—	—	
Sept. 11, 1929.....	+	+	+	—	—	—	
Sept. 24, 1929.....	+	+	+	—	—	—	
Oct. 8, 1929.....	+	+	+	—	—	—	
Oct. 21, 1929.....	+	+	P	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929.....	+	+	+	—	—	—	
Nov. 19, 1929.....	+	+	+	—	—	—	
Dec. 19, 1929.....	+	+	+	—	—	—	
Jan. 8, 1930.....	+	+	P	—	—	—	
Jan. 22, 1930.....	+	+	+	S	—	—	
Feb. 5, 1930.....	+	+	+	—	—	—	
Feb. 19, 1930.....	+	+	+	—	—	—	
Mar. 5, 1930.....	+	+	P	—	—	—	Apr. 11, 1930, produced a vigorous calf; guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum did not acquire abortion disease.
Mar. 19, 1930.....	+	+	+	—	—	—	
Apr. 2, 1930.....	+	+	—	—	—	—	
July 16, 1930.....	+	+	P	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1491

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 2, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	+	
June 4, 1929.....	+	+	+	+	+	+	
June 18, 1929.....	+	+	+	+	+	+	
July 2, 1929.....	+	+	+	+	+	—	
July 17, 1929.....	+	+	+	+	+	—	July 20, 1929, bred.
July 31, 1929.....	+	+	+	+	—	—	Aug. 10, 1929, bred.
Aug. 27, 1929.....	+	+	S	—	—	—	
Sept. 11, 1929.....	+	+	+	—	—	—	Oct. 31, 1929, bred.
Sept. 24, 1929.....	+	+	+	—	—	—	
Oct. 8, 1929.....	+	+	+	—	—	—	
Oct. 21, 1929.....	+	+	+	—	—	—	Dec. 7, 1929, bred. Jan. 10, 1930, bred. Feb. 8, 1930, bred.
Nov. 6, 1929.....	+	+	+	—	—	—	
Nov. 19, 1929.....	+	+	S	—	—	—	
Dec. 19, 1929.....	+	+	+	S	—	—	June 10, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Jan. 8, 1930.....	+	+	+	P	—	—	
Feb. 5, 1930.....	+	+	+	+	—	—	
Mar. 5, 1930.....	+	+	P	—	—	—	Nov. 6, 1930, produced a vigorous calf (271 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Apr. 2, 1930.....	+	+	+	—	—	—	
Apr. 16, 1930.....	+	+	+	—	—	—	
June 11, 1930.....	+	+	+	P	S	—	Jan. 28, 1931, milk negative for <i>Br. abortus</i> .
June 17, 1930.....	+	+	+	P	—	—	
June 28, 1930.....	+	+	+	+	—	—	
July 16, 1930.....	+	+	+	+	—	—	Nov. 6, 1930, produced a vigorous calf (271 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Aug. 19, 1930.....	+	+	+	S	—	—	
Oct. 2, 1930.....	+	+	+	—	—	—	
Nov. 11, 1930.....	+	+	+	—	—	—	Jan. 28, 1931, milk negative for <i>Br. abortus</i> .
Nov. 22, 1930.....	+	+	P	—	—	—	
Jan. 9, 1931.....	+	+	P	—	—	—	

## VACCINATED HEIFER 1499

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 23, 1929.....	—	—	—	—	—	P	
Apr. 30, 1929.....	+	+	+	+	+	—	
May 2, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	P	—	
June 4, 1929.....	+	+	+	+	—	—	
June 18, 1929.....	+	+	+	P	—	—	
July 2, 1929.....	+	+	+	P	—	—	June 21, 1929, bred.
July 17, 1929.....	+	+	+	+	—	—	
July 31, 1929.....	+	+	+	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Aug. 27, 1929.....	+	+	S	—	—	—	
Sept. 11, 1929.....	+	+	+	—	—	—	
Sept. 24, 1929.....	+	+	S	—	—	—	Mar. 28, 1930, produced a vigorous calf (280 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum failed to acquire abortion disease.
Oct. 8, 1929.....	+	P	—	—	—	—	
Oct. 21, 1929.....	+	+	—	—	—	—	
Nov. 6, 1929.....	+	+	+	—	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Nov. 19, 1929.....	+	+	+	—	—	—	
Dec. 19, 1929.....	+	+	+	—	—	—	
Jan. 8, 1930.....	+	+	S	—	—	—	Mar. 28, 1930, produced a vigorous calf (280 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum failed to acquire abortion disease.
Jan. 22, 1930.....	+	+	+	—	—	—	
Feb. 5, 1930.....	+	+	+	—	—	—	
Feb. 19, 1930.....	+	+	P	—	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Mar. 5, 1930.....	+	+	P	—	—	—	
Mar. 19, 1930.....	+	+	—	—	—	—	
Apr. 2, 1930.....	+	+	—	—	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Apr. 16, 1930.....	+	P	—	—	—	—	
Apr. 22, 1930.....	+	S	—	—	—	—	
July 16, 1930.....	+	+	P	—	—	—	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Aug. 19, 1930.....	+	+	P	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1528

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	P	—	—	—	—	—	
May 2, 1929.....	+	P	—	—	—	—	
May 7, 1929.....	+	+	+	+	+	—	
May 14, 1929.....	+	+	+	+	+	—	
May 21, 1929.....	+	+	+	+	+	—	
June 4, 1929.....	+	+	+	+	+	—	
June 18, 1929.....	+	+	+	P	—	—	
July 2, 1929.....	+	P	P	—	—	—	
July 17, 1929.....	+	+	P	—	—	—	
July 31, 1929.....	+	P	—	—	—	—	
Aug. 27, 1929.....	P	S	—	—	—	—	
Sept. 11, 1929.....	+	S	—	—	—	—	
Sept. 24, 1929.....	+	—	—	—	—	—	
Oct. 8, 1929.....	+	—	—	—	—	—	
Oct. 21, 1929.....	P	—	—	—	—	—	
Nov. 6, 1929.....	P	—	—	—	—	—	
Nov. 19, 1929.....	P	—	—	—	—	—	
Dec. 19, 1929.....	P	—	—	—	—	—	
Jan. 8, 1930.....	P	—	—	—	—	—	Feb. 10, 1930, bred.
Feb. 5, 1930.....	S	—	—	—	—	—	
Mar. 5, 1930.....	—	—	—	—	—	—	June 10, 1930, received <i>Br. abortus</i> , conjunctival exposure. Nov. 20, 1930, produced a vigorous calf (283 days); guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease. Jan. 31, 1931, milk negative for <i>Br. abortus</i> .
Apr. 2, 1930.....	—	—	—	—	—	—	
June 11, 1930.....	+	—	—	—	—	—	
June 17, 1930.....	P	—	—	—	—	—	
June 28, 1930.....	+	+	+	S	—	—	
July 16, 1930.....	+	+	+	S	—	—	
Oct. 2, 1930.....	+	+	—	—	—	—	
Nov. 11, 1930.....	P	S	—	—	—	—	
Nov. 22, 1930.....	—	—	—	—	—	—	

## VACCINATED HEIFER 1532

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine ( <i>Br. abortus</i> strain 484).
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	—	—	—	—	
May 2, 1929.....	+	+	—	—	—	—	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	P	S	
May 21, 1929.....	+	+	+	+	+	—	
June 4, 1929.....	+	+	+	+	P	—	
June 18, 1929.....	+	+	+	+	—	—	
July 2, 1929.....	+	P	S	—	—	—	
July 17, 1929.....	P	P	—	—	—	—	
July 31, 1929.....	—	—	—	—	—	—	
Aug. 27, 1929.....	P	S	—	—	—	—	
Sept. 11, 1929.....	S	—	—	—	—	—	
Sept. 24, 1929.....	P	—	—	—	—	—	
Oct. 8, 1929.....	—	—	—	—	—	—	
Oct. 21, 1929.....	P	—	—	—	—	—	
Nov. 6, 1929.....	—	—	—	—	—	—	
Nov. 19, 1929.....	—	—	—	—	—	—	
Dec. 19, 1929.....	—	—	—	—	—	—	
Jan. 8, 1930.....	P	—	—	—	—	—	Mar. 18, 1930, bred.
Feb. 5, 1930.....	—	—	—	—	—	—	
Mar. 5, 1930.....	—	—	—	—	—	—	June 10, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Apr. 2, 1930.....	—	—	—	—	—	—	
Apr. 16, 1930.....	—	—	—	—	—	—	Dec. 15, 1930, produced a vigorous calf (272 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum did not acquire abortion disease. Mar. 20, 1931, milk negative for <i>Br. abortus</i> .
June 11, 1930.....	+	+	—	—	—	—	
June 17, 1930.....	P	—	—	—	—	—	
June 28, 1930.....	+	+	P	—	—	—	
July 16, 1930.....	+	+	+	—	—	—	
Aug. 19, 1930.....	P	—	—	—	—	—	
Oct. 2, 1930.....	—	—	—	—	—	—	
Nov. 11, 1930.....	S	—	—	—	—	—	
Jan. 9, 1931.....	S	—	—	—	—	—	

TABLE 5.—Records of individual experimental animals—Continued

## CONTROL HEIFER 1427

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Oct. 8, 1929.....	—	—	—	—	—	—	May 27, 1929, bred.
Oct. 21, 1929.....	—	—	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929.....	+	P	—	—	—	—	
Nov. 19, 1929.....	+	+	—	—	—	—	
Dec. 19, 1929.....	+	+	+	+	+	—	
Jan. 8, 1930.....	+	+	+	+	+	—	
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Feb. 19, 1930.....	+	+	+	+	+	+	Feb. 26, 1930, produced a vigorous calf (275 days); guinea pigs inoculated with uterine material and guinea pigs inoculated with colostrum acquired abortion disease.
Mar. 5, 1930.....	+	+	+	+	+	+	
Mar. 19, 1930.....	+	+	+	+	+	+	
Apr. 2, 1930.....	+	+	+	+	+	P	
Apr. 15, 1930.....	+	+	+	+	+	+	
July 16, 1930.....	+	+	+	+	+	+	
Aug. 19, 1930.....	+	+	+	P	—	—	Aug. 16, 1930, milk positive for <i>Br. abortus</i> .
Oct. 22, 1930.....	+	+	+	P	—	—	Aug. 22, 1930, milk positive for <i>Br. abortus</i> .
Nov. 11, 1930.....	+	+	P	S	—	—	
Jan. 9, 1931.....	+	+	+	S	—	—	

## CONTROL HEIFER 1429

Oct. 8, 1929.....	—	—	—	—	—	—	June 6, 1929, bred.
Oct. 21, 1929.....	—	—	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929.....	+	+	+	+	+	—	
Nov. 19, 1929.....	+	+	+	+	P	—	
Dec. 19, 1929.....	+	+	+	+	+	+	
Jan. 8, 1930.....	+	+	+	+	+	+	Jan. 10, 1930, aborted (218 days); guinea pigs were infected with abortion disease by the inoculation of uterine material and colostrum.
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Feb. 19, 1930.....	+	+	+	+	+	P	
Mar. 5, 1930.....	+	+	+	+	+	P	
Mar. 19, 1930.....	+	+	+	+	+	S	

## CONTROL HEIFER 1447

Oct. 8, 1929.....	—	—	—	—	—	—	May 8, 1929, bred.
Oct. 21, 1929.....	—	—	—	—	—	—	June 21, 1929, bred.
Nov. 6, 1929.....	+	+	+	S	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 19, 1929.....	+	+	+	+	+	—	
Dec. 19, 1929.....	+	+	+	+	+	—	
Jan. 8, 1930.....	+	+	+	+	+	+	Nov. 26, 1929, aborted (158 days); guinea pigs infected with abortion disease by inoculation of uterine material and colostrum.
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Mar. 19, 1930.....	+	+	+	+	+	+	

## CONTROL HEIFER 1489

Oct. 8, 1929.....	—	—	—	—	—	—	June 21, 1929, bred.
Oct. 21, 1929.....	—	—	—	—	—	—	Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 6, 1929.....	+	+	P	—	—	—	
Nov. 19, 1929.....	+	+	+	—	—	—	
Dec. 19, 1929.....	+	+	+	+	+	+	Dec. 15, 1929, aborted (177 days); <i>Br. abortus</i> isolated from uterine material and colostrum through guinea-pig inoculations.
Jan. 8, 1930.....	+	+	+	+	+	+	
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	
Feb. 19, 1930.....	+	+	+	+	+	+	
Mar. 5, 1930.....	+	+	+	+	+	+	
Mar. 19, 1930.....	+	+	+	+	+	+	
Apr. 2, 1930.....	+	+	+	+	+	+	
Apr. 16, 1930.....	+	+	+	+	+	+	
May 7, 1930.....	+	+	+	+	+	+	
May 17, 1930.....	+	+	+	+	+	+	
July 16, 1930.....	+	+	+	+	+	+	
Aug. 19, 1930.....	+	+	+	+	+	+	

TABLE 5.—Records of individual experimental animals—Continued

## CONTROL HEIFER 1548

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Oct. 8, 1929.....	—	—	—	—	—	—	Breeding date not recorded. Oct. 19, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Oct. 21, 1929.....	—	—	—	—	—	—	
Nov. 6, 1929.....	+	+	+	+	P	—	
Nov. 19, 1929.....	+	+	+	+	—	—	
Dec. 19, 1929.....	+	+	+	+	P	—	
Jan. 8, 1930.....	+	+	+	+	—	—	Feb. 15, 1930, aborted; <i>Br. abortus</i> isolated from uterine material and from colostrum through guinea-pig inoculations.
Jan. 22, 1930.....	+	+	+	+	+	—	
Feb. 5, 1930.....	+	+	+	+	+	—	
Feb. 19, 1930.....	+	+	+	+	+	+	
Mar. 5, 1930.....	+	+	+	+	+	P	
Mar. 19, 1930.....	+	+	+	+	+	+	
Apr. 2, 1930.....	+	+	+	+	+	+	
Apr. 16, 1930.....	+	+	+	+	+	+	
May 7, 1930.....	+	+	+	+	+	+	
May 17, 1930.....	+	+	+	+	+	P	
July 16, 1930.....	+	+	+	+	—	—	
Aug. 19, 1930.....	+	+	+	+	S	—	

## CONTROL HEIFER 1446

Jan. 28, 1930.....	—	—	—	—	—	—	Sept. 3, 1929, bred.
Mar. 5, 1930.....	+	+	+	+	+	—	Feb. 24, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Mar. 19, 1930.....	+	+	+	+	+	P	
Apr. 2, 1930.....	+	+	+	+	+	+	
Apr. 16, 1930.....	+	+	+	+	+	+	Mar. 17, 1930, aborted (195 days); guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.
May 7, 1930.....	+	+	+	+	+	+	
July 16, 1930.....	+	+	+	+	P	P	
Aug. 19, 1930.....	+	+	+	+	+	+	
Jan. 9, 1931.....	+	+	+	+	+	+	

## CONTROL HEIFER 1625

June 4, 1930.....	—	—	—	—	—	—	Apr. 2, 1930, bred.
June 10, 1930.....	—	—	—	—	—	—	June 10, 1930, received <i>Br. abortus</i> , conjunctival exposure.
June 17, 1930.....	P	—	—	—	—	—	
June 28, 1930.....	+	+	+	+	P	P	
July 16, 1930.....	+	+	+	+	+	+	
Aug. 19, 1930.....	+	+	+	+	+	+	
Oct. 2, 1930.....	+	+	+	+	+	+	Sept. 6, 1930, aborted (157 days); guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.
Nov. 11, 1930.....	+	+	+	+	+	+	
Jan. 9, 1931.....	+	+	+	+	+	+	

## CONTROL HEIFER 1584

Oct. 2, 1930.....	—	—	—	—	—	—	Dec. 5, 1929, bred.
Oct. 22, 1930.....	—	—	—	—	—	—	Apr. 3, 1930, bred.
Nov. 10, 1930.....	+	+	+	+	P	—	July 16, 1930, bred.
Dec. 20, 1930.....	+	+	+	+	S	—	Oct. 22, 1930, received <i>Br. abortus</i> , conjunctival exposure.
Jan. 9, 1931.....	+	+	+	+	+	+	
Feb. 19, 1931.....	+	+	+	+	+	+	Jan. 13, 1931, aborted (181 days); guinea pigs inoculated with uterine material and with colostrum acquired abortion disease.

Although the vaccine injections caused the development of marked agglutination reactions in the treated animals, the reactions in the course of time became much reduced in titer. It was not unusual for the agglutination titers of the principals to increase considerably for a few weeks after the conjunctival exposures and then to subside before parturition.



## DISCUSSION OF RESULTS

The results of the experiments were regarded as encouraging. If an attempt had not been made to obtain information as to the possibilities of the intradermic method of vaccine administration, the final results in all probability would have been even more favorable because implantation of the vaccine in the udder seemed to occur more regularly after intradermic than after subcutaneous injections. Moreover, strain 484 was plainly too virulent for use in vaccine, for the three cows which received, either intradermically or subcutaneously, vaccine prepared from this strain, became long-time carriers of the infection in their milk.

None of the *Brucella abortus* strains used for the preparation of vaccine in these experiments were regarded as avirulent. Although the vaccine prepared from strain 11, the least virulent one employed, produced no visible lesions, in the 17 guinea pigs into which it was injected either subcutaneously or intra-abdominally, strain 11 on other occasions caused moderately marked lesions in guinea pigs that were killed two months after injection. Pregnant, susceptible cows that received subcutaneous administrations of vaccine prepared from this strain have, in some cases, maintained serological reactions of such intensity as to indicate localization of the infection in their udders. As the experiments proceeded and further guinea-pig inoculation tests were made of strains 11 and 19, some doubt arose as to whether there was a considerable degree of difference between the virulence of these two strains, the immunizing results of which appeared to be very much alike in cattle.

The results of the experiments failed to indicate that the three *Brucella abortus* strains used in the preparation of the vaccines differed to any marked degree in immunizing effect. From the standpoint of safety, however, it was plainly evident that they differed markedly. Vaccine prepared from as virulent a strain as No. 484 could reasonably be expected to become implanted in the udder of many cows, even if the vaccine were subcutaneously administered when the cows were nonpregnant. Consequently the use of such a strain would be highly objectionable. That strain 19 was more virulent than strain 11 for cattle was evidenced by the fact that the intradermic injection of the former into a nonpregnant cow caused her to eliminate the microorganism in her milk for a prolonged period, whereas after the administration of the latter in the same manner, the infection could not with certainty be detected in the milk at any time. This conclusion was based on the development of moderately marked agglutination reactions of guinea pigs after they were inoculated with milk rather than on the development of abortion disease lesions in these animals. Vaccines prepared from strains 11 and 19, when administered subcutaneously, gave evidence of being noninfective for the udders of nonpregnant cows.

The degree of *Brucella abortus* exposure to which the animals in this experiment were subjected was severe, as is shown by the fact that seven of eight controls had agglutination reactions with titers of 1 to 100 or higher in from 9 to 18 days after exposure. The single exception required a 2-month interval to react with a titer of 1 to 200 but with a titer of 1 to 50 she reacted at the end of 18 days.

No appreciable difference was observed in the immunizing results produced by a 10 c c and a 20 c c dose of abortion vaccine of a density 10 times that of tube 1 of the McFarland nephelometer, when the doses were administered subcutaneously. It seems reasonable to believe that, with a suspension of the density employed, the amount injected could be subjected to even much greater variation without observable differences in results.

The intensity of the thermic reactions that were occasioned by the vaccine injections did not appear to furnish any reliable information as to the degree of immunity which the vaccine might be expected to confer; in fact, the variation in such reactions shown by different animals was slight.

### CONCLUSIONS

The data which the experiment yielded seem to justify the following conclusions:

In the preparation of abortion vaccine for use in nonpregnant cattle, the virulence of the *Brucella abortus* strain or strains from which the vaccine is prepared is a matter of great importance, particularly when the vaccine is to be used as an immunizing agent for nonpregnant cows with functioning udders.

The virulence of *Brucella abortus* strains for cattle can be determined with a considerable degree of accuracy by their effects on guinea pigs.

Abortion vaccine prepared from strains of *Brucella abortus* sufficiently virulent to cause extensive lesions in guinea pigs is likely to implant the infection in the udders of nonpregnant cows and may be eliminated in the milk for long periods.

Abortion vaccine prepared from strains of *Brucella abortus* of greatly reduced virulence, as indicated by guinea-pig-inoculation results, gives evidence of being reasonably efficient as an immunizing agent for abortion-disease-free cows and heifers when the vaccine is administered subcutaneously two months or more before service. The danger of such a vaccine being implanted in the udder of the vaccinated animal seems to be slight.

There appears to be more danger of causing infection of the udder, in which it may persist for a long time, if the vaccine is injected intradermically than if it is injected subcutaneously; hence, the former seems to be a less satisfactory method of administration. The conjunctival method of exposure regularly transmits abortion disease to cattle.

# EFFICACY OF AN AVIRULENT STRAIN OF *BRUCELLA ABORTUS* FOR VACCINATING PREGNANT CATTLE<sup>1</sup>

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## INTRODUCTION

The experiment here discussed is one of a series conducted to develop, if possible, improved means of combating infectious abortion in cattle. This paper, dealing with the vaccination phase of the study, reports a continuation of investigations conducted in 1927 and 1928, the results of which were presented briefly in 1928<sup>2</sup> and in more detail in 1932.<sup>3</sup> The present experiment makes use of a different method of exposure and a larger number of animals.

Previous work at the Experiment Station,<sup>4</sup> Bethesda, Md., showed that a strain of *Brucella abortus* that had become so attenuated, through long cultivation, that it would no longer produce lesions in guinea pigs unless administered in very large doses, would confer a considerable degree of resistance against virulent strains. The use of a similar strain by Huddleson<sup>5</sup> not only induced immunity in guinea pigs, but also afforded a considerable degree of protection to cattle. Furthermore, Huddleson found that the strain he employed could be given to pregnant cows and heifers with impunity.

Although the investigations of Huddleson led him to conclude that the particular avirulent strain with which he worked increased resistance whether given before or after breeding, the results obtained at the Experiment Station indicated that the avirulent strain there used did not induce sufficient immunity, when given before conception, to protect the animals against a severe exposure, but if administered after conception it offered some protection.

The need for a dependable method of treating pregnant, non-infected animals in infected herds and the encouragement afforded by the results of Huddleson and by those obtained at the Experiment Station prompted further investigation of this phase of the immunization problem.

## VACCINE USED AND TESTS FOR VIRULENCE

The *Brucella abortus* strain used in this experiment, designated *Br. abortus* strain 801, was isolated in 1915 from the milk of an infected cow. When the cattle in the experiment were vaccinated, this strain had undergone from 182 to 193 transfers. Despite the fact that it had lived so long on culture media and had given indica-

<sup>1</sup> Received for publication May 26, 1932; issued March, 1933.

<sup>2</sup> MOHLER, J. R. Rpt. of the Chief of the Bur. Anim. Indus., U. S. Dept. Agr. Ann. Rpt., 20-22. 1928.

<sup>3</sup> COTTON, W. E. EFFICACY OF DIFFERENT STRAINS OF *BRUCELLA ABORTUS* AS IMMUNIZING AGENTS AGAINST INFECTIOUS ABORTION. Jour. Agr. Research 45: 705-724. 1932.

<sup>4</sup> SCHROEDER, E. C., and COTTON, W. E. RECENT BUREAU OF ANIMAL INDUSTRY EXPERIMENT STATION BOVINE INFECTIOUS ABORTION STUDIES. Jour. Amer. Vet. Med. Assoc. 66: 550-561. 1925.

<sup>5</sup> HUDDLESON, I. F. THE VACCINAL IMMUNIZATION OF ANIMALS AGAINST BACTERIUM ABORTUS (BANG) INFECTION. U. S. Livestock Sanit. Assoc. Proc. (1925) 29: 210-215. 1926.

tion of being nonlesion producing for guinea pigs and nonpathogenic for cattle, its agglutinating value had never seemed to be impaired.

To prove its lack of virulence for guinea pigs, groups of these animals were inoculated at irregular intervals from August 17, 1928, to October 14, 1929, with physiological salt solution suspensions of the microorganisms that had been transferred on culture media from 162 to 193 times. Of the 91 guinea pigs used, 73 were injected subcutaneously and 18 intra-abdominally. The suspensions used in making the inoculations had a density equal to 10 times that of tube 1 of the McFarland nephelometer; the subcutaneous dose was  $\frac{1}{4}$  c c and the intra-abdominal dose was  $1\frac{1}{2}$  c c.

Six of the guinea pigs were killed and autopsied at the end of six weeks, but the remainder were allowed to live about two months after they were inoculated. No lesions were observed, on autopsy, in any of the guinea pigs except testicle adhesions in six of those that were inoculated intra-abdominally. These lesions were not considered as being characteristic of abortion disease but rather as the result of a large amount of foreign material injected into the peritoneal cavity.

*Brucella abortus* was isolated from the spleens of 2 of the 6 guinea pigs that were killed at the end of six weeks after inoculation, though these organs showed no abortion-disease lesions. It was not possible, however, to isolate this microorganism from the spleens of 12 other guinea pigs which were permitted to live two months after inoculation.

The avirulent *Brucella abortus* suspensions, though incapable of causing lesions to develop in any of the 91 guinea pigs, nevertheless induced the formation of agglutinins in the blood of all but a small proportion of them. At the time of autopsy a blood sample was taken from each guinea pig. These samples on being tested for *Br. abortus* agglutinins gave the following results:

- 11 negative in all dilutions, 1-25 to 1-500
- 7 positive in dilutions of 1-25
- 15 positive in dilutions of 1-50
- 17 positive in dilutions of 1-100
- 19 positive in dilutions of 1-200
- 22 positive in dilutions of 1-500 or more

The variations in reactions were not confined to any particular group of guinea pigs.

One nonreacting heifer, about five months pregnant, was subjected to an intravenous injection of strain 801 as a preliminary test of its virulence on cattle. This heifer, No. 1336, was bred September 4, 1928. On February 4, 1929, 10 c c of a suspension of strain 801, about twice the density of tube 1 of the McFarland nephelometer, was introduced into one of her jugular veins. Serum agar slants that were sown with a loopful of the material used for the intravenous exposure developed a heavy growth of *Brucella abortus*. Blood serum drawn from the heifer on February 4, just before the intravenous injection, gave negative results to the agglutination test. An agglutination test of her blood serum made March 23, about seven weeks after the injection, showed a titer of 1 to 500. On May 14 the titer had decreased to 1 to 200. The heifer gave birth to a vigorous calf June 17, 1929, 286 days after breeding. Her blood serum then showed slight agglutination in a 1 to 100 dilution.

Her placenta came away promptly. Six guinea pigs which were inoculated with an emulsion of cotyledons, and six guinea pigs which were inoculated with a composite sample of colostrum, were free from evidence of abortion disease when they were killed two months later. Although but one bovine animal was used at this time for determining the virulence of the strain on cattle, the test was considered as being a severe one in view of the fact that the intravenous method was used in introducing the infection.

#### EXPERIMENT ANIMALS AND METHODS OF VACCINATION

To determine the value of this avirulent strain, administered after conception, in inducing resistance to *Brucella abortus*, use was made of 19 pregnant cows and heifers. Of these 10 were principals and 9 were controls. Five principals, cows 1503 and 1506 and heifers 1497, 1527, and 1534, were vaccinated April 23, 1929; two principals, cow 1494 and heifer 1424, were vaccinated May 11, 1929; and three principals, heifers 1547, 1549, and 1556, were vaccinated October 1, 1929, and again on October 15, 1929.

A large number of the animals used in the experiment were pregnant when purchased, hence no information was available as to their service dates. They were subjected to the tube agglutination test for infectious abortion at the time of purchase. The results were negative in all cases. Examinations of the animals made a few days before they were vaccinated indicated that they were between two and four months pregnant.

The vaccine administered to the five principals on April 23, 1929, was prepared from the one hundred and eighty-second transfer of strain 801. The vaccine administered to the two principals on May 11, 1929, was prepared from the one hundred and eighty-seventh transfer of the strain, and the vaccine administered to the three animals on October 1, 1929, and October 15, 1929, was prepared from the one hundred and ninety-second and one hundred and ninety-third transfers.

The vaccine in all cases was adjusted to a density of about 10 times that of tube 1 of the McFarland nephelometer. Of the animals which were vaccinated but once, four received a 20 c c dose and three received a 10 c c dose. The three animals vaccinated twice received 10 c c of the vaccine on each occasion. When a 20 c c dose was used, half the quantity was injected under the skin in front of each shoulder, but when a 10 c c dose was used, the material was introduced at only one point.

The temperatures of all the vaccinated animals were taken just before the vaccine was injected and daily thereafter for several days. The vaccine injections caused a rise in temperature of 3° to 4° by the second or third day, but by the fourth or fifth day the temperature had usually become normal again. Swellings which appeared at the points of inoculation sometimes reached a diameter of 3 to 4 inches and a thickness of from one-half to three-fourths of an inch by the second or third day, but within a week after the injection they had practically disappeared.

## TESTS FOR BRUCELLA ABORTUS IN MILK OF VACCINATED ANIMALS

Samples of milk were obtained from cows 1503, 1506, and 1494 a week after vaccination and at weekly intervals thereafter until nine samples had been obtained from each animal. Four guinea pigs were then inoculated intra-abdominally with 5 c c of a composite sample to determine, if possible, whether *Brucella abortus* had invaded the udders of the cows. Although the development of abortion-disease lesions could hardly be anticipated, even if abortion micro-organisms were present in the milk, it seemed reasonable to expect that if present they would cause reactions in the blood serum of the guinea pigs. In no instance, however, were the results of the blood-serum agglutination tests found to be positive when the guinea pigs were killed about two months after inoculation, nor could *Br. abortus* lesions be detected in the guinea pigs at autopsy.

## EXPOSURE OF EXPERIMENT ANIMALS TO BRUCELLA ABORTUS

The vaccinated cattle were exposed to virulent strains of *Brucella abortus* within one to two months after vaccination. Seven principals and seven controls were exposed June 26, 1929, and three principals and two controls were exposed November 18, 1929.

At the time of the earlier exposure, it was not realized that the conjunctival method of exposure regularly transmits abortion disease to cattle, consequently both the conjunctival and ingestion methods were used. However, when the second lot of animals were exposed only the conjunctival method was used. Suspensions of *Br. abortus* were employed for the exposures in all cases. The suspensions used on June 26, 1929, were prepared from two virulent strains of *Br. abortus*, Nos. 484 and 1420. Both represented the sixth transfer. For the conjunctival exposure, a suspension twenty times the density of tube 1 of the McFarland nephelometer was used. From three to four drops were deposited on the conjunctiva of one eye of each animal. For the ingestion exposure, each animal received 100 c c of a suspension, the density of which was about twice that of the McFarland nephelometer tube 1. The three principals and two controls which were exposed on November 18, 1929, received a suspension prepared from the second, fourth, and sixth transfers, respectively, of three virulent *Br. abortus* strains, Nos. 1497, 1456, and 1531. Four drops of the suspension, which was about twenty times the density of the McFarland nephelometer tube 1, were deposited on the conjunctiva of one eye of each animal.

When an animal calved or aborted, six guinea pigs were inoculated with uterine discharges or placental material. Six guinea pigs were also inoculated with 5 c c of a composite sample of colostrum. When animals aborted, cultural studies were made of the fetuses. Table 1 shows the immunizing results of the 19 animals used in the experiment.

TABLE 1.—*Immunizing results obtained by the use of Brucella abortus 801 strain vaccine in 10 principals as compared with the results obtained with 9 controls*

## ANIMALS VACCINATED WITH BRUCELLA ABORTUS 801 STRAIN VACCINE

Animal No.	Date of vaccination	Vaccine dose	Date of exposure	Date of calving or aborting	Outcome of pregnancy	Results of uterine examinations for <i>Br. abortus</i> <sup>a</sup>	Results of colostrum examinations for <i>Br. abortus</i> <sup>a</sup>
Cow: 1503	Apr. 23, 1929	c c 20	June 26, 1929	Sept. 29, 1929	Vigorous calf	—	—
Heifer: 1497	do	20	do	Aug. 19, 1929	Aborted	+	+
Cow: 1506	do	20	do	Sept. 4, 1929	Weak calf	+	+
Heifer: 1527	do	10	do	Dec. 6, 1929	Vigorous calf	—	—
1534	do	10	do	Aug. 10, 1929	Aborted	+	+
Cow: 1494	May 11, 1929	20	do	Nov. 9, 1929	Vigorous calf	—	—
Heifer: 1424	do	10	do	Oct. 9, 1929	Weak calf	+	+
1547	{ Oct. 1, 1929 Oct. 15, 1929	10 10	Nov. 18, 1929	Feb. 24, 1930	Vigorous calf	—	—
1549	{ Oct. 1, 1929 Oct. 15, 1929	10 10			Aborted	+	+
1556	{ Oct. 1, 1929 Oct. 15, 1929	10 10	do	Mar. 13, 1930	Weak calf (died)	+	+

## CONTROL ANIMALS NOT VACCINATED

Cow: 1376			June 26, 1929	July 26, 1929	Aborted	+	+
Heifer: 1450			do	Nov. 21, 1929	Vigorous calf	—	—
1538			do	Aug. 25, 1929	Aborted	+	+
1540			do	Oct. 26, 1929	do	+	+
Cow: 1488			do	Aug. 28, 1929	do	+	+
Heifer: 1531			do	Sept. 17, 1929	do	+	+
1486			do	Sept. 30, 1929	do	+	—
1553			Nov. 18, 1929	Feb. 21, 1930	do	+	+
1554			do	Jan. 5, 1930	Weak calf (died)	+	+

<sup>a</sup> Key: + indicates presence of *Brucella abortus*; — indicates absence of *Br. abortus*.

Of the 10 vaccinated animals which were exposed to virulent strains of *Brucella abortus*, 4 produced vigorous calves. The presence of *Br. abortus* in the uterus or colostrum at the time of calving could not be demonstrated. Three principals produced weak calves, one of which died shortly after birth. *Br. abortus* was found in the uterus and colostrum of each of these cows. Three principals aborted, and *Br. abortus* was isolated from the uterus and colostrum of each of them.

Of the nine control animals only one produced a vigorous calf. Her uterus and colostrum appeared to be free from *Brucella abortus* infection. One control animal produced a calf which did not have sufficient vigor to live. *Br. abortus* infection was demonstrated in the uterus and colostrum of the dam. Seven of the control animals aborted. *Br. abortus* infection was found in the uteri of all seven animals and in the colostrum of six.

In the group of 10 vaccinated animals the placenta was promptly expelled by 8 animals and retained by 2. In the group of 9 control animals the placenta was expelled by 3 animals and retained by 6.

Table 2 gives in detail the records of the 19 animals used in the experiment.

TABLE 2.—Records of individual experimental animals <sup>a</sup>

## VACCINATED COW 1503

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used <sup>b</sup>						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection 20 c c abortion vaccine.
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 30, 1929, milk negative for <i>Br. abortus</i> .
Apr. 30, 1929.....	+	+	+	+	P	P	May 7, 1929, milk negative for <i>Br. abortus</i> .
May 7, 1929.....	+	+	+	+	+	—	May 14, 1929, milk negative for <i>Br. abortus</i> .
May 14, 1929.....	+	+	+	+	+	—	May 21, 1929, milk negative for <i>Br. abortus</i> .
May 21, 1929.....	+	+	+	+	+	—	May 28, 1929, milk negative for <i>Br. abortus</i> .
June 4, 1929.....	+	+	+	+	—	—	June 4, 1929, milk negative for <i>Br. abortus</i> .
June 11, 1929.....	+	+	+	+	—	—	June 18, 1929, milk negative for <i>Br. abortus</i> .
June 18, 1929.....	+	+	+	+	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
June 27, 1929.....	+	+	P	—	—	—	July 2, 1929, milk negative for <i>Br. abortus</i> .
July 2, 1929.....	+	+	+	P	—	—	Sept. 21, 1929, milk negative for <i>Br. abortus</i> .
July 17, 1929.....	+	+	+	+	+	—	Sept. 29, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and colostrum failed to acquire abortion disease.
July 31, 1929.....	+	+	+	+	P	—	Oct. 15, 1929, milk negative for <i>Br. abortus</i> .
Aug. 27, 1929.....	+	+	+	S	—	—	
Sept. 11, 1929.....	+	+	+	—	—	—	
Oct. 8, 1929.....	+	+	+	—	—	—	
Oct. 23, 1929.....	+	P	—	—	—	—	
Nov. 6, 1929.....	+	+	—	—	—	—	
Nov. 19, 1929.....	+	P	—	—	—	—	
Dec. 19, 1929.....	+	P	—	—	—	—	
Jan. 8, 1930.....	+	P	—	—	—	—	
Jan. 22, 1930.....	+	S	—	—	—	—	
Feb. 5, 1930.....	+	+	—	—	—	—	
Mar. 5, 1930.....	+	+	P	—	—	—	
Apr. 2, 1930.....	+	S	—	—	—	—	
July 16, 1930.....	+	P	S	—	—	—	
Oct. 2, 1930.....	+	P	S	—	—	—	
Nov. 11, 1930.....	+	P	S	—	—	—	

## VACCINATED HEIFER 1497

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine.
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	P	
May 14, 1929.....	+	+	+	+	+	—	
May 21, 1929.....	+	+	+	+	P	—	
June 4, 1929.....	+	+	+	+	—	—	
June 11, 1929.....	+	+	+	+	—	—	
June 18, 1929.....	+	+	+	+	—	—	
June 27, 1929.....	+	+	+	P	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
July 2, 1929.....	+	+	+	+	—	—	
July 17, 1929.....	+	+	+	+	—	—	
July 31, 1929.....	+	+	+	+	+	+	Aug. 19, 1929, aborted; <i>Br. abortus</i> isolated from fetus by cultural methods; guinea pigs inoculated with uterine material and with colostrum developed abortion disease.
Aug. 27, 1929.....	+	+	+	+	+	+	Oct. 15, 1929, milk positive for <i>Br. abortus</i> .
Sept. 11, 1929.....	+	+	+	+	+	+	
Sept. 24, 1929.....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929.....	+	+	+	+	+	+	
Nov. 6, 1929.....	+	+	+	+	+	+	

## VACCINATED COW 1506

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 20 c c abortion vaccine.
Apr. 23, 1929.....	—	—	—	—	—	—	Apr. 30, 1929, milk negative for <i>Br. abortus</i> .
Apr. 30, 1929.....	+	+	+	+	+	+	May 7, 1929, milk negative for <i>Br. abortus</i> .
May 7, 1929.....	+	+	+	+	+	P	May 14, 1929, milk negative for <i>Br. abortus</i> .
May 14, 1929.....	+	+	+	+	+	—	May 21, 1929, milk negative for <i>Br. abortus</i> .
May 21, 1929.....	+	+	+	P	—	—	May 28, 1929, milk negative for <i>Br. abortus</i> .
June 4, 1929.....	+	+	+	+	—	—	June 4, 1929, milk negative for <i>Br. abortus</i> .
June 11, 1929.....	+	+	+	+	—	—	June 11, 1929, milk negative for <i>Br. abortus</i> .
June 18, 1929.....	+	+	+	+	—	—	June 18, 1929, milk negative for <i>Br. abortus</i> .
June 27, 1929.....	+	+	+	P	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
July 2, 1929.....	+	+	+	P	—	—	
July 31, 1929.....	+	+	+	+	+	+	

<sup>a</sup> Key: + indicates pronounced clumping of bacteria; P, partial clumping; S, a trace of agglutination; —, no evidence of clumping.

<sup>b</sup> Figures at head of columns indicate cubic centimeters of blood serum. They represent approximate dilutions of 1 to 25, 1 to 50, 1 to 100, 1 to 200, 1 to 500, and 1 to 1,000, respectively, with the amount of antigen (1 c c) used.



TABLE 2.—Records of individual experimental animals—Continued

## VACCINATED COW 1506—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Aug. 27, 1929.....	+	+	+	+	S	—	July 2, 1929, milk negative for <i>Br. abortus</i> . Sept. 4, 1929, produced a weak calf; guinea pigs inoculated with uterine material and with colostrum acquired abortion disease. Sept. 24, 1929, milk negative for <i>Br. abortus</i> . Oct. 15, 1929, milk negative for <i>Br. abortus</i> .
Sept. 11, 1929.....	+	+	+	+	+	+	
Sept. 24, 1929.....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929.....	+	+	+	+	+	P	
Nov. 6, 1929.....	+	+	+	+	+	+	
Nov. 19, 1929.....	+	+	+	+	P	—	
Dec. 19, 1929.....	+	+	+	+	—	—	
Jan. 8, 1930.....	+	+	+	P	—	—	
Jan. 22, 1930.....	+	+	+	P	—	—	
Feb. 5, 1930.....	+	+	+	P	—	—	
Mar. 5, 1930.....	+	+	+	—	—	—	
Apr. 2, 1930.....	+	+	+	—	—	—	
June 16, 1930.....	+	+	+	P	—	—	
							Jan. 19, 1931, milk negative for <i>Br. abortus</i> .

## VACCINATED HEIFER 1527

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine.
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	+	+	+	+	+	+	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	+	
May 21, 1929.....	+	+	+	+	+	—	
June 4, 1929.....	+	+	+	+	—	—	
June 11, 1929.....	+	+	+	+	—	—	
June 18, 1929.....	+	+	+	+	P	—	
June 27, 1929.....	+	+	+	+	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
July 2, 1929.....	+	+	P	P	—	—	
July 17, 1929.....	+	+	+	+	—	—	
July 31, 1929.....	+	+	+	+	—	—	
Aug. 27, 1929.....	+	+	S	—	—	—	
Sept. 11, 1929.....	+	+	+	+	—	—	
Oct. 8, 1929.....	+	+	P	—	—	—	
Oct. 23, 1929.....	+	+	+	—	—	—	
Nov. 6, 1929.....	+	+	+	—	—	—	
Nov. 19, 1929.....	+	+	P	—	—	—	Dec. 6, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not contract abortion disease.
Dec. 19, 1929.....	+	+	P	—	—	—	
Jan. 8, 1930.....	+	+	—	—	—	—	
Jan. 22, 1930.....	+	+	P	—	—	—	
Feb. 5, 1930.....	+	+	+	—	—	—	
Mar. 5, 1930.....	+	+	—	—	—	—	
Apr. 2, 1930.....	+	P	—	—	—	—	

## VACCINATED HEIFER 1534

Apr. 10, 1929.....	—	—	—	—	—	—	Apr. 23, 1929, subcutaneous injection of 10 c c abortion vaccine.
Apr. 23, 1929.....	—	—	—	—	—	—	
Apr. 30, 1929.....	—	—	—	—	—	—	
May 7, 1929.....	+	+	+	+	+	+	
May 14, 1929.....	+	+	+	+	+	—	
May 21, 1929.....	+	+	+	+	—	—	
June 4, 1929.....	+	+	+	P	—	—	
June 11, 1929.....	+	+	+	—	—	—	
June 18, 1929.....	+	+	P	—	—	—	
June 27, 1929.....	+	+	+	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
July 2, 1929.....	+	+	P	—	—	—	
July 17, 1929.....	+	+	+	+	P	—	
July 31, 1929.....	+	+	+	+	—	—	
Aug. 27, 1929.....	+	+	+	+	+	S	Aug. 10, 1929, aborted; guinea pigs which were inoculated with uterine material and with colostrum contracted abortion disease.
Sept. 11, 1929.....	+	+	+	+	+	+	
Sept. 24, 1929.....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929.....	+	+	+	+	+	+	
Nov. 6, 1929.....	+	+	+	P	P	P	
Nov. 19, 1929.....	+	+	+	+	+	+	
Jan. 22, 1930.....	+	+	+	+	+	S	
Feb. 5, 1930.....	+	+	+	+	—	—	
							Jan. 21, 1930, milk negative for <i>Br. abortus</i> . Feb. 15, 1930, milk negative for <i>Br. abortus</i> .

VACCINATED COW 1494

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
May 7, 1929	-	-	-	-	-	-	May 11, 1929, subcutaneous injection of 20 c c abortion vaccine.
May 14, 1929	-	-	-	-	-	-	May 21, 1929, milk negative for <i>Br. abortus</i> .
May 21, 1929	+	+	+	+	+	+	May 28, 1929, milk negative for <i>Br. abortus</i> .
June 4, 1929	+	+	+	+	+	+	June 4, 1929, milk negative for <i>Br. abortus</i> .
June 11, 1929	+	+	+	+	+	+	June 11, 1929, milk negative for <i>Br. abortus</i> .
June 18, 1929	+	+	+	+	+	+	June 18, 1929, milk negative for <i>Br. abortus</i> .
June 27, 1929	+	+	+	+	+	+	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
July 2, 1929	+	+	+	+	+	+	July 2, 1929, milk negative for <i>Br. abortus</i> .
July 7, 1929	+	+	+	+	+	-	Aug. 13, 1929, milk negative for <i>Br. abortus</i> .
July 31, 1929	+	+	+	+	+	P	Sept. 21, 1929, milk negative for <i>Br. abortus</i> .
Aug. 27, 1929	+	+	P	S	-	-	Nov. 9, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not contract abortion disease.
Sept. 11, 1929	+	+	+	P	-	-	Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Sept. 24, 1929	+	+	+	P	-	-	
Oct. 8, 1929	+	+	+	P	-	-	
Oct. 23, 1929	+	+	P	-	-	-	
Nov. 6, 1929	+	+	P	-	-	-	
Nov. 19, 1929	+	+	+	P	-	-	

May 7, 1929	—	—	—	—	—	May 11, 1929, subcutaneous injection of 10 c c abortion vaccine.
May 14, 1929	—	—	—	—	—	
May 21, 1929	+	+	+	+	+	
June 4, 1929	+	+	+	P	S	
June 11, 1929	+	+	+	+	—	
June 18, 1929	+	+	+	+	—	
June 27, 1929	+	+	P	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
July 2, 1929	+	+	+	—	—	
July 17, 1929	+	+	+	—	—	
July 31, 1929	+	+	+	—	—	
Aug. 27, 1929	+	+	+	—	P	
Sept. 11, 1929	+	+	+	—	+	
Sept. 24, 1929	+	+	+	—	+	
Oct. 8, 1929	+	+	+	—	+	Oct. 9, 1929, produced a weak calf; guinea pigs inoculated with uterine material and with colostrum contracted abortion disease.
Oct. 23, 1929	+	+	+	—	+	
Nov. 6, 1929	+	+	+	—	+	
Nov. 19, 1929	+	+	+	+	+	

Sept. 11, 1929	—	—	—	—	—	—	Oct. 1, 1929, subcutaneous injection of 10 c c abortion vaccine.
Sept. 24, 1929	—	—	—	—	—	—	
Oct. 8, 1929	+	+	+	+	+	+	Oct. 15, 1929, subcutaneous injection of 10 c c abortion vaccine.
Oct. 23, 1929	—	—	—	—	—	S	
Nov. 6, 1929	+	+	+	+	+	+	Nov. 18, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 19, 1929	+	+	+	+	+	P	
Dec. 19, 1929	+	+	+	+	P	—	
Jan. 8, 1930	+	+	+	—	—	—	Feb. 24, 1930, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not contract abortion disease.
Jan. 22, 1930	+	+	+	—	—	—	
Feb. 5, 1930	+	+	+	S	S	—	
Feb. 19, 1930	+	+	+	S	—	—	
Mar. 5, 1930	+	+	—	—	—	—	
Mar. 19, 1930	+	+	—	—	—	—	
Apr. 2, 1930	+	+	—	—	—	—	
July 16, 1930	+	+	—	—	—	—	

Sept. 11, 1929	—	—	—	—	—	Oct. 1, 1929, subcutaneous injection of 10 c c abortion vaccine.
Sept. 24, 1929	—	—	—	—	—	Oct. 15, 1929, subcutaneous injection of 10 c c abortion vaccine.
Oct. 8, 1929	+	+	+	P	S	Nov. 18, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Oct. 23, 1929	+	+	+	+	+	Jan. 27, 1930, aborted; <i>Br. abortus</i> isolated from both uterine material and colostrum through inoculation of guinea pigs.
Nov. 6, 1929	+	+	+	+	+	
Nov. 19, 1929	+	+	+	+	+	
Dec. 19, 1929	+	+	+	+	+	
Jan. 8, 1930	+	+	+	+	+	
Jan. 22, 1930	+	+	+	+	+	
Feb. 5, 1930	+	+	+	+	+	
Feb. 19, 1930	+	+	+	+	+	
Mar. 5, 1930	+	+	+	+	+	
Mar. 19, 1930	+	+	+	+	+	
Apr. 2, 1930	+	+	+	+	+	
May 17, 1930	+	+	+	+	+	

TABLE 2.—Records of individual experimental animals—Continued

## VACCINATED HEIFER 1556

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Sept. 11, 1929.....	—	—	—	—	—	—	Oct. 1, 1929, subcutaneous injection of 10 c c abortion vaccine. Oct. 15, 1929, subcutaneous injection of 10 c c abortion vaccine. Nov. 18, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Sept. 24, 1929.....	—	—	—	—	—	—	
Oct. 8, 1929.....	+	+	+	P	S	—	
Oct. 23, 1929.....	+	+	+	+	+	+	
Nov. 6, 1929.....	+	+	+	+	+	—	
Nov. 19, 1929.....	+	+	+	+	—	—	
Dec. 19, 1929.....	+	+	+	+	+	+	
Jan. 8, 1930.....	+	+	+	+	+	+	
Jan. 22, 1930.....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	—	
Feb. 19, 1930.....	+	+	+	+	S	—	Mar. 13, 1930, produced a weak calf which died; guinea pigs inoculated with uterine material and colostrum contracted abortion disease. Aug. 19, 1930, milk negative for <i>Br. abortus</i> .
Mar. 5, 1930.....	+	+	+	+	—	—	
Mar. 19, 1930.....	+	+	+	+	+	P	
Apr. 2, 1930.....	+	+	+	+	+	+	
July 16, 1930.....	+	+	P	S	—	—	
Aug. 19, 1930.....	+	+	+	—	P	—	
Oct. 2, 1930.....	+	+	+	+	+	—	

## CONTROL COW 1376

Apr. 10, 1929.....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929.....	—	—	—	—	—	—	
June 11, 1929.....	—	—	—	—	—	—	
June 27, 1929.....	—	—	—	—	—	—	
July 2, 1929.....	—	—	—	—	—	—	July 26, 1929, aborted; guinea pigs inoculated with uterine material and with colostrum contracted abortion disease.
July 17, 1929.....	+	+	+	+	+	—	
July 31, 1929.....	+	+	+	+	S	—	
Aug. 27, 1929.....	+	+	+	+	+	—	
Sept. 11, 1929.....	+	+	+	+	+	+	Oct. 15, 1929, milk positive for <i>Br. abortus</i> .
Sept. 24, 1929.....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929.....	+	+	+	+	+	+	
Nov. 6, 1929.....	+	+	+	+	+	+	Jan. 21, 1930, milk positive for <i>Br. abortus</i> .
Nov. 19, 1929.....	+	+	+	+	+	+	
Jan. 22, 1930.....	+	+	+	+	+	S	
Feb. 5, 1930.....	P	P	P	P	—	—	

## CONTROL HEIFER 1450

Apr. 10, 1929.....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929.....	P	—	—	—	—	—	
June 11, 1929.....	S	—	—	—	—	—	
June 27, 1929.....	—	—	—	—	—	—	
July 2, 1929.....	—	—	—	—	—	—	
July 17, 1929.....	+	P	—	—	—	—	
July 31, 1929.....	+	P	—	—	—	—	
Aug. 27, 1929.....	S	—	—	—	—	—	
Sept. 11, 1929.....	—	—	—	—	—	—	
Sept. 24, 1929.....	—	—	—	—	—	—	
Oct. 8, 1929.....	—	—	—	—	—	—	Nov. 21, 1929, produced a vigorous calf; guinea pigs inoculated with uterine material and with colostrum did not acquire abortion disease.
Oct. 23, 1929.....	S	—	—	—	—	—	
Nov. 6, 1929.....	—	—	—	—	—	—	
Nov. 19, 1929.....	—	—	—	—	—	—	
Jan. 8, 1930.....	—	—	—	—	—	—	
Jan. 22, 1930.....	—	—	—	—	—	—	
Feb. 5, 1930.....	S	—	—	—	—	—	

## CONTROL HEIFER 1538

Apr. 10, 1929.....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929.....	—	—	—	—	—	—	
June 11, 1929.....	—	—	—	—	—	—	
June 27, 1929.....	—	—	—	—	—	—	
July 2, 1929.....	—	—	—	—	—	—	
July 17, 1929.....	+	+	+	+	+	+	
July 31, 1929.....	+	+	+	+	+	+	

TABLE 2.—Records of individual experimental animals—Continued

## CONTROL HEIFER 1538—Continued

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Aug. 27, 1929....	+	+	+	+	+	S	Aug. 25, 1929, aborted; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
Sept. 11, 1929....	+	+	+	+	+	+	
Sept. 24, 1929....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929....	+	+	+	+	+	—	Oct. 15, 1929, milk positive for <i>Br. abortus</i> .
Nov. 6, 1929.....	+	+	+	+	+	—	
Nov. 19, 1929....	+	+	+	+	+	—	
Jan. 22, 1930....	+	+	+	+	+	+	Jan. 21, 1930, milk positive for <i>Br. abortus</i> .
Feb. 5, 1930.....	+	+	+	+	+	+	Feb. 15, 1930, milk positive for <i>Br. abortus</i> .

## CONTROL HEIFER 1540

Apr. 10, 1929....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929....	—	—	—	—	—	—	
June 11, 1929....	—	—	—	—	—	—	
June 27, 1929....	—	—	—	—	—	—	
July 2, 1929.....	—	—	—	—	—	—	
July 17, 1929....	+	+	+	+	—	—	
July 31, 1929....	+	+	+	+	—	—	
Aug. 27, 1929....	+	+	+	+	+	S	Oct. 26, 1929, aborted; <i>Br. abortus</i> isolated from uterine material and colostrum through guinea-pig inoculations.
Sept. 11, 1929....	+	+	+	+	+	+	
Sept. 24, 1929....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929....	+	+	+	+	+	+	
Nov. 6, 1929.....	+	+	+	+	+	+	
Nov. 19, 1929....	+	+	+	+	+	+	

## CONTROL COW 1488

Apr. 10, 1929....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929....	—	—	—	—	—	—	
June 11, 1929....	—	—	—	—	—	—	
June 27, 1929....	—	—	—	—	—	—	
July 2, 1929.....	—	—	—	—	—	—	Aug. 28, 1929, aborted; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
July 17, 1929....	+	+	+	+	+	—	
July 31, 1929....	+	+	+	+	+	+	
Aug. 27, 1929....	+	+	+	+	+	S	
Sept. 11, 1929....	+	+	+	+	+	+	Jan. 21, 1930, milk positive for <i>Br. abortus</i> . Feb. 15, 1930, milk positive for <i>Br. abortus</i> .
Sept. 24, 1929....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929....	+	+	+	+	+	+	
Nov. 6, 1929.....	+	+	+	+	+	+	
Nov. 19, 1929....	+	+	+	+	+	+	
Jan. 22, 1930....	+	+	+	+	+	+	
Feb. 5, 1930.....	+	+	+	+	+	+	

## CONTROL HEIFER 1531

Apr. 10, 1929....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929....	—	—	—	—	—	—	
June 11, 1929....	—	—	—	—	—	—	
June 27, 1929....	—	—	—	—	—	—	
July 2, 1929.....	—	—	—	—	—	—	
July 17, 1929....	+	+	+	+	—	—	
July 31, 1929....	+	+	+	+	—	—	
Aug. 27, 1929....	+	+	+	+	S	—	Sept. 17, 1929, aborted; guinea pigs inoculated with uterine material and colostrum contracted abortion disease.
Sept. 11, 1929....	+	+	+	+	+	+	
Sept. 24, 1929....	+	+	+	+	+	+	
Oct. 8, 1929.....	+	+	+	+	+	+	
Oct. 23, 1929....	+	+	+	+	+	P	Oct. 15, 1929, milk positive for <i>Br. abortus</i> .
Nov. 6, 1929.....	+	+	+	+	P	P	
Nov. 19, 1929....	+	+	+	P	P	P	

TABLE 2.—Records of individual experimental animals—Continued

## CONTROL HEIFER 1456

Date	Agglutination results when quantity of blood serum (cubic centimeters) indicated was used						Remarks
	0.04	0.02	0.01	0.005	0.002	0.001	
Apr. 10, 1929....	—	—	—	—	—	—	June 26, 1929, received <i>Br. abortus</i> , conjunctival and ingestion exposure.
May 21, 1929....	—	—	—	—	—	—	
June 11, 1929....	—	—	—	—	—	—	
June 27, 1929....	—	—	—	—	—	—	
July 2, 1929....	—	—	—	—	—	—	
July 17, 1929....	+	+	+	+	+	—	Sept. 30, 1929, aborted; <i>Br. abortus</i> isolated from uterus through guinea-pig inoculations, but guinea pigs inoculated with colostrum failed to acquire abortion disease. Oct. 15, 1929, milk negative for <i>Br. abortus</i> . Jan. 21, 1930, milk negative for <i>Br. abortus</i> .
July 31, 1929....	+	+	+	+	—	—	
Aug. 27, 1929....	+	+	+	—	—	—	
Sept. 11, 1929....	+	+	+	+	+	—	
Sept. 24, 1929....	+	+	+	+	+	+	
Oct. 8, 1929....	+	+	+	+	P	—	
Oct. 23, 1929....	+	+	+	+	+	+	
Nov. 6, 1929....	+	+	+	+	+	+	
Nov. 19, 1929....	+	+	+	+	+	—	
Jan. 22, 1930....	+	+	+	+	—	P	
Feb. 5, 1930....	+	+	+	+	P	—	
Apr. 2, 1930....	+	+	+	+	—	—	
Apr. 16, 1930....	+	+	—	—	—	—	

## CONTROL HEIFER 1553

Nov. 6, 1929....	—	—	—	—	—	—	Nov. 18, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 19, 1929....	—	—	—	—	—	—	
Dec. 19, 1929....	+	+	+	+	—	—	
Jan. 8, 1930....	+	+	+	+	—	—	
Jan. 22, 1930....	+	+	+	+	P	—	
Feb. 5, 1930....	+	+	+	+	+	+	Feb. 21, 1930, aborted; guinea pigs inoculated with uterine material and with colostrum contracted abortion disease.
Feb. 19, 1930....	+	+	+	+	+	+	
Mar. 5, 1930....	+	+	+	+	+	+	
Mar. 19, 1930....	+	+	+	+	+	+	
Apr. 2, 1930....	+	+	+	+	+	+	
Apr. 16, 1930....	+	+	+	+	+	+	Aug. 16, 1930, milk positive for <i>Br. abortus</i> . Oct. 21, 1930, milk positive for <i>Br. abortus</i> .
May 7, 1930....	+	+	+	+	+	+	
July 16, 1930....	+	+	+	+	+	+	
Aug. 19, 1930....	+	+	+	+	+	+	

## CONTROL HEIFER 1554

Nov. 6, 1929....	—	—	—	—	—	—	Nov. 18, 1929, received <i>Br. abortus</i> , conjunctival exposure.
Nov. 19, 1929....	—	—	—	—	—	—	
Dec. 19, 1929....	+	+	+	+	—	—	Jan. 5, 1930, produced a weak calf which died; guinea pigs inoculated both with uterine material and with colostrum acquired abortion disease.
Jan. 8, 1930....	+	+	+	+	—	—	
Jan. 22, 1930....	+	+	+	+	+	+	
Feb. 5, 1930....	+	+	+	+	+	+	
Feb. 19, 1930....	+	+	+	+	+	+	
Mar. 5, 1930....	+	+	+	+	+	+	
Mar. 19, 1930....	+	+	+	+	+	+	
Apr. 2, 1930....	+	+	+	+	+	+	
Apr. 16, 1930....	+	+	+	+	+	+	
May 7, 1930....	+	+	+	+	+	+	
July 16, 1930....	+	+	+	+	+	+	
Aug. 19, 1930....	+	+	+	+	+	+	

## DISCUSSION OF RESULTS

The experiment indicates that the use of vaccine prepared from an avirulent *Brucella abortus* strain (No. 801), administered during pregnancy, could not be depended upon to confer a pronounced degree of immunity against abortion disease. On the other hand, the vaccine may be considered as having exerted some beneficial effects, for whereas 40 per cent of the principals withstood the degree of *Br.*

*abortus* exposure to which they were subjected, only 11 per cent of the control animals resisted it; and whereas 89 per cent of the control animals aborted or produced weak calves, only 60 per cent of the vaccinated animals had similar histories.

The *Brucella abortus* exposure given in the experiment was sufficiently severe to cause all but one of the nine control animals to develop marked reactions to the abortion agglutination test and either to abort or to produce weak calves which did not survive. Agglutination tests were not made with sufficient frequency to determine the exact length of time that elapsed between *Br. abortus* exposure and the appearance of agglutinins in the blood. The tests made, however, showed that six of the nine control animals acquired agglutination titers of from 1 to 100 to 1 to 1,000 in 21 days. Two controls acquired 1 to 200 titers in 31 days. The control that calved normally failed to develop a titer of more than 1 to 50 after exposure to *Br. abortus*. This was the only animal of the 19 used in the experiment which, previous to being subjected to artificial *Br. abortus* exposure, had had a titer of 1 to 25. It is possible that some earlier natural exposure to the disease might have been responsible for the immunity which she manifested in the experiment.

Failure of the vaccinated cattle to resist more generally the exposure to which they were subjected may have been due in part to the fact that a comparatively brief interval, from one to two months, elapsed between the vaccination dates and the dates of exposure to *Brucella abortus*. In view of the nature of the earlier results obtained with this avirulent strain, it seems possible that a considerable period must elapse after vaccination before a serviceable degree of immunity can reasonably be expected. Whether vaccination in abortion disease is followed by a negative phase of resistance and, if so, how long this phase continues, are matters on which few data have been accumulated.

#### SUMMARY

To determine the efficacy of an avirulent strain of *Brucella abortus* for vaccinating pregnant cattle against abortion disease, vaccines were prepared and administered subcutaneously to three pregnant cows and seven pregnant heifers.

The strain of *Brucella abortus* used for the vaccines was isolated in 1915 from the milk of an infected cow and had undergone from 182 to 193 transfers on artificial culture media when the vaccines were prepared.

Inoculation tests showed the strain, designated as No. 801, to be nonpathogenic for cattle and guinea pigs. The agglutinating value of the strain, however, was not impaired.

From one to two months after vaccination 10 experiment animals were exposed to virulent strains of *Brucella abortus*; 9 other animals were used as controls.

The vaccines gave evidence of conferring immunity against abortion disease to 40 per cent of the vaccinated animals; only 11 per cent of the control animals resisted the disease.

No evidence was obtained to indicate that the strain becomes localized in the udders of vaccinated animals.

# BIOLOGY OF THE FLOUR BEETLES, *TRIBOLIUM CONFUSUM* DUV. AND *T. FERRUGINEUM* FAB.<sup>1</sup>

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## INTRODUCTION

The flour beetles have long been known to be among the more serious pests of flour, meal, and other cereal products. Numerous references in the literature give ample evidence of their importance as destroyers of these foodstuffs. However, owing probably to the rather long adult life of these insects and the difficulties in observing the immature stages, no thorough work has been done on their life history. Several suppositions of early authors regarding the life cycle have been copied and recopied until now these statements, often erroneous, are accepted as facts. The following preliminary account is a summary of rather extensive experiments on these two species commenced by the writer in the latter part of 1929.

## DIFFERENTIATING CHARACTERS OF ADULTS

The two species *Tribolium confusum* Duv. and *T. ferrugineum* Fab. are so similar in size, color, shape of body, and habits that they are continually confused, even by experienced entomologists. The distinguishing characters are so constant, however, that once these are learned there can never be any doubt as to their identity. The character found most useful by the writer for distinguishing the two species is the size of the eyes on the ventral surface of the head. In *T. ferrugineum* the width of each eye is approximately equal to the distance separating them on the underside of the head. In *T. confusum* the eyes, viewed from below, appear small, the width of each eye being approximately only one-third that of the distance separating them. This character can be used for the identification of living specimens, whereas the character usually given—i. e., the sudden enlargement of the last three joints of the antennae in *T. ferrugineum* and the gradual enlargement in *T. confusum*—is almost useless for identifying living specimens because the antennae are usually in motion. Another differentiating trait, useful for field identification but rather unreliable, is that when placed on a flat surface under a strong light *T. ferrugineum* often attempts to fly, and sometimes makes short flights of a few feet, while *T. confusum* never makes any attempt at flight.

Careful measurements of 50 adults show them to be somewhat smaller than the measurements usually given. *Tribolium confusum* is found to average 3.47 mm in length and 1.07 mm in width through the prothorax. These measurements are almost identical with those

<sup>1</sup> Received for publication June 6, 1932; issued March, 1933.

given by Brindley,<sup>2</sup> but are considerably smaller than those of Chapman<sup>3</sup> and other authors. *T. ferrugineum* averages 3.32 mm in length and 1.03 mm in width through the prothorax.

#### ORIGIN, HABITAT, AND DISTRIBUTION

The origin of insects infesting stored products is difficult to determine because they are widely distributed by commerce and most of them have long been cosmopolitan pests. In the case of *Tribolium ferrugineum* a hint as to its probable origin is given by Blair,<sup>4</sup> who refers to it under the name *T. castaneum* Hbst. He shows that in India this species is commonly found in the wild state under bark and in wood. It is also found in such situations in North America and elsewhere, but not at all frequently. It is an insect of subtropical climates, and very likely India is its original home. This view is strengthened by the fact that, in the same article, Blair has described a very closely related species, *T. indicum*, from the same country and with the same habitat, which does not occur in stored products.

As to the original habitat of the genus, there can be no reasonable doubt that before the advent of civilized man it lived under bark and in old logs. Here it was probably a scavenger, but this can not be definitely proved. Almost without exception, the beetles of the subfamily Ulominae, of which *Tribolium* is a member, occur either as pests of stored products or else under the bark of trees and in rotting logs. It seems evident that all the members of this group originally lived in the latter habitat and have recently adopted the flour-feeding habit. Two species of *Tribolium*, *T. madens* Charp. and *T. indicum* Blair, are found almost exclusively in such situations, and the two flour pests *T. confusum* and *T. ferrugineum* are themselves occasionally found there.

These two species are known to be cosmopolitan, occurring throughout the world in stored cereal products. Temperature seems to influence their distribution somewhat. *Tribolium ferrugineum* is essentially an insect of warm climates, being seldom taken north of the fortieth parallel. *T. confusum*, on the other hand, seems to prefer cooler climates and is most abundant in the northern part of the United States.

#### FOOD

A wide variety of foodstuffs are attacked by *Tribolium*, a list of which is given by Chittenden.<sup>5</sup> Practically any kind of flour, meal, breakfast food, and cracked grain is attacked, and much damage is done to dried insect specimens in collections. The writer has found these pests as much to be feared in insect collections as the dermestids. They are also known to breed in certain spices. Three other preferred foods which seem to have received little or no mention are chocolate, raisins, and various nuts, especially Persian (English) walnuts, on which the larvae develop very rapidly.

<sup>2</sup> BRINDLEY, T. A. THE GROWTH AND DEVELOPMENT OF EPHESTIA KUEHNIELLA ZELLER (LEPIDOPTERA) AND TRIBOLIUM CONFUSUM DUVAL (COLEOPTERA) UNDER CONTROLLED CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY. Ann. Ent. Soc. Amer. 23: 741-757, illus. 1930.

<sup>3</sup> CHAPMAN, R. N. THE CONFUSED FLOUR BEETLE (TRIBOLIUM CONFUSUM DUVAL). Minn. State Ent. Rpt. 17: 73-94, illus. 1918.

<sup>4</sup> BLAIR, K. G. THE INDIAN SPECIES OF PALORUS, MULS. (COLEOPTERA: TENEBRIONIDAE) AND SOME ASSOCIATED BEETLES. Indian Forest Rec. 14 (5): 1-20 (133-152), illus. 1930.

<sup>5</sup> CHITTENDEN, F. H. INSECTS AFFECTING CEREALS AND OTHER DRY VEGETABLE FOODS. Chapter 8, in The Principal Household Insects of the United States. U. S. Dept. Agr., Div. Ent. Bul. (n. s.) 4: 112-131, illus. 1896.



## OVERWINTERING

In heated flour mills, warehouses, etc., *Tribolium* breed the year around and all stages can be found at any time during the year. In unheated flour mills this is not the case. Some authors have stated that these beetles do not live over winter in unheated mills but that these mills are reinfested in the spring from neighboring heated mills. An inspection of several unheated flour mills in Maryland and northern Virginia during February, 1931, revealed many adults of *T. confusum* in a semidormant condition, but no living larvae or pupae. As the adults usually live a year, or over, it is evident that in unheated flour mills in this territory the winter is passed in the adult stage and breeding begins with the approach of spring. In the Gulf States breeding probably continues the year around, while in the extreme Northern States and Canada the species may not be able to survive the winter except in heated buildings. *T. ferrugineum* seems to be less resistant to cold than *T. confusum*.

## LONGEVITY

It has been known that *Tribolium* adults are long-lived, but no actual tests have previously been made to determine the maximum length of life. Estimates have ranged from three months to a year or more. As many of the adults under observation by the writer are still alive, no definite statement can yet be made as to the maximum length of life except that it is considerably longer than is generally supposed. Of 50 individuals of *T. confusum* emerging nearly 24 months previous to this writing (January, 1932), 13 are still alive and active. *T. ferrugineum* appears to be somewhat shorter lived than *T. confusum*, but 5 individuals out of an original 60 are still alive after 23 months.

## OVIPOSITION

Of all phases of the life history of *Tribolium*, oviposition seems to have received the least attention. Only two investigators, Chapman and Brindley, appear to have done any work on the oviposition of these insects, and they have been concerned only with the number of eggs laid per day by young females and have made no attempt to determine the total number of eggs laid or the length of the oviposition period. Inasmuch as the oviposition period may last for more than a year and it is almost impossible to locate the eggs in the flour, it is not surprising that more work has not been done along this line.

In the experiments made by the writer 25 pairs of each species were segregated on emergence and placed in different foods under different conditions of temperature and humidity. Small vials, lightly stoppered with cotton, were used as containers. A single pair of adults was placed in each vial and moved to another vial every day. Various methods of locating the eggs were tried, but it was found that the method, first advocated by Chapman, of counting the larvae rather than the eggs was more accurate. As approximately 90 per cent of the eggs hatched, the actual number of eggs laid is about one-ninth greater than the figures given, which concern viable eggs only.

Table 1 gives the average duration of the egg-laying period and the average number of eggs laid for each group of females used in the experiments.

TABLE 1.—Summary of data concerning oviposition of *Tribolium confusum* and *T. ferrugineum*

## T. CONFUSUM

Temperature	Food	Females		Average oviposition period		Average eggs laid per day	
		Number	Days	Number	Number	Number	Number
27° C.	Whole-wheat flour	6	214	521			2.43
	Bran	6	263	333			1.26
	Oatmeal	2	136	141			1.04
Room	White flour	3	325	187			.58
	Whole-wheat flour	7	280	744			2.66

## T. FERRUGINEUM

27° C.	Whole-wheat flour	6	171	438			2.56
	Middlings	3	102	246			2.41
	Bran	3	147	235			1.60
Room	Whole-wheat flour	3	160	518			3.24
	Corn meal	3	228	500			2.19
	Middlings	2	143	302			2.11
	Oatmeal	3	245	123			.50
	White flour	2	73	19			.26

The longest oviposition period observed was that of a female of *Tribolium confusum* in whole-wheat flour at room temperature. This female laid viable eggs for 432 days. The longest period for *T. ferrugineum* was 308 days, for a female that was kept in oatmeal at room temperature. The greatest number of viable eggs laid was 976, by a female of *T. confusum* also kept in whole-wheat flour at room temperature. The greatest number of viable eggs laid by *T. ferrugineum* was 956; this individual was kept in corn meal at room temperature. The number of eggs laid per day is not large. In no case were more than 13 viable eggs laid in one day by a single female, and the average was only 2 or 3 per day. Under optimum conditions Brindley<sup>6</sup> records 18 eggs in one day and a much higher daily average than is indicated here.

Mating was observed rather frequently among the pairs of adults in the experiment. The writer's records show that the female may continue to lay viable eggs for as long as five months after being separated from the male.

## THE EGG STAGE

The eggs are usually laid singly, directly in the flour, but occasionally they are found attached to the sides of the container. They are coated with a sticky substance which causes the flour to adhere to them. The length of the incubation period varies considerably with the external conditions. Fifty eggs of *Tribolium ferrugineum* kept in an incubator at 27° C. hatched in an average period of 5.5 days. Forty eggs kept at room temperature averaged 8.6 days. Here the temperature ranged from 18.5° to 28.5° and averaged 22°, while the humidity ranged from 22 to 43 per cent and averaged 32 per cent.

The incubation period for *Tribolium confusum* is slightly longer. Forty eggs kept in an incubator at 27° C. hatched in an average period of 6.8 days. Of these, 17 kept in continuous light averaged 6.5 days,

<sup>6</sup>Brindley, T. A. Op. cit.

and 23 kept in continuous darkness averaged 7 days. Forty eggs kept at room temperature required on an average 12.8 days to hatch. In this case the temperature ranged from 14.5° to 26° and averaged 21°, while the relative humidity ranged from 24 to 51 per cent and averaged 34 per cent.

#### THE LARVAL PERIOD

Chapman<sup>7</sup> and Brindley<sup>8</sup> found six larval instars in *Tribolium confusum*. Through continued observation the writer has determined that there is no fixed number of larval molts, but that the number ranges from 6 to 11 or more and is normally 7 or 8 instead of 6. This variation is due both to external conditions, such as food, temperature, and humidity, and to individual characteristics entirely apart from external influences.

The number of larval instars was determined by actual count of the number of times individual larvae molted before pupation. Over 100 eggs of both species of *Tribolium* were placed in individual containers and observed each day until the individuals emerged as adults. Varying conditions of food, temperature, and humidity were used. After each molt the exuviae could be seen in the small vial used as a container and were immediately removed and recorded.

Table 2 gives a summary of experiments to determine the number and duration of the larval instars under various conditions.

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<sup>7</sup> Chapman, R. N. Op. cit.

<sup>8</sup> Brindley, T. A. Op. cit.

TABLE 2.—Summary of data on the larval period of *Tribolium confusum* and of *T. ferrugineum*

## T. CONFUSUM

Temperature	Food	Larvae	Average duration of each larval instar <sup>a</sup>											Entire larval period
			First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	Eleventh	
27° C.	(Middlings.....)	Number	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days
	(Bran.....)	4	1.6	4.5	4.0	4.2	4.5	4.5	6.5	5.0(1)	---	---	---	31.0
	(Oatmeal.....)	11	2.2	5.7	6.5	6.4	6.5	6.6	6.7	7.3(7)	---	---	---	45.7
	(White flour.....)	5	2.0	5.8	6.8	8.2	8.0	13.2	8.4	9.4	8.0(1)	---	---	64.6
		8	2.0	4.9	7.5	7.5	12.0	16.5	14.2(5)	19.7(3)	---	---	---	80.0(3)

## T. FERRUGINEUM

27° C.	(Bran.....)	7	2.0	4.4	4.0	4.1	4.1	6.1	7.0(4)	7.0(1)	---	---	---	28.9
	(Whole-wheat flour.....)	7	1.7	4.3	4.0	4.1	4.3	4.9	6.1	7.0(1)	---	---	---	30.4
	(Corn meal.....)	8	2.0	4.6	5.0	4.0	4.5	6.1	7.6(5)	7.3(3)	---	---	---	31.9
	(Middlings.....)	7	2.0	4.7	4.3	4.0	4.3	6.0	7.0(4)	8.5(2)	---	---	---	32.6
Room.	(Oatmeal.....)	7	1.9	5.6	7.9	8.9	10.6	11.1	9.5(6)	26.0(1)	14.0(1)	9.0(1)	---	68.2(4)
	(White flour.....)	7	2.0	5.6	5.9	9.4	18.0	21.7(3)	17.0(1)	14.0(1)	---	---	---	87.5(2)
	(Middlings.....)	7	2.3	7.9	6.0	4.9	4.6	7.6	12.7(3)	13.0(2)	---	---	---	46.8
	(Whole-wheat flour.....)	0	2.2	8.3	6.5	4.8	4.5	7.2	11.0(5)	6.0(1)	11.0(1)	---	---	51.5
Room.	(Bran.....)	4	2.7	9.2	8.3	6.3	5.3	6.0	12.5	10.7(6)	9.6(1)	---	---	64.9
	(Corn meal.....)	7	2.6	10.6	9.0	7.0	8.9	10.1	14.0	21.0(2)	20.0(1)	---	---	79.8(3)
	(White flour.....)	4	2.3	8.5	12.0	14.2	21.5	20.5	8.3	8.0	9.7	11.7	15.7	114.0
	(Oatmeal.....)	3	2.7	10.0	12.0	11.3	12.7	12.0	8.3	---	---	---	---	---

<sup>a</sup> Numbers in parenthesis indicate number of individuals passing through any molt if less than original number.

There is considerable variation in the length of the larval period due both to the kind of food and to the temperature. Whole wheat flour, middlings, bran, and corn meal are all acceptable foods. Growth is very slow in white flour and many of the larvae die before reaching the adult stage. Under certain conditions Persian walnuts have been found to be very favorable for larval development, some of the shortest life cycles being recorded with this food. A constant temperature of 27° C. proved to be much more suitable for development than ordinary room temperatures. In Table 2 the room temperatures were those found in the laboratory at Washington, D. C., during April and May.

There is considerable individual variation but practically no overlapping of the measurements of an individual of one instar with those of the next. The following tabulation shows the average widths of the head capsules of 40 larvae of *Tribolium confusum* of the different instars:

Instar	Width (mm) of head capsule	Instar	Width (mm) of head capsule
First.....	0. 175	Sixth.....	0. 459
Second.....	. 197	Seventh.....	. 585
Third.....	. 249	Eighth.....	. 619
Fourth.....	. 311	Ninth.....	. 655
Fifth.....	. 387		

#### THE PUPAL PERIOD

When ready to pupate, the mature larva comes to the surface of the food in which it has been working and, after a short prepupal period, transforms to the naked pupa, lying in or on the surface of the food without protection of any kind. The vacated pupal cells of the Mediterranean flour moth are often found to contain several *Tribolium* pupae.

The average duration of the pupal period was found to be as follows:

Thirty-one *T. confusum* pupae kept at 27° C. in continuous darkness emerged as adults in from 7 to 12 days, with an average of 8.74 days.

Forty-three *T. confusum* pupae kept at 27° C. in continuous light emerged as adults in from 6 to 9 days, with an average of 7.86 days.

One hundred and twelve *T. ferrugineum* pupae kept at 27° C., for the most part in continuous darkness, had a pupal period of from 6 to 9 days, with an average of 7.14 days.

Thirty-two *T. ferrugineum* pupae kept at room temperature during the early summer had a pupal period of from 5 to 14 days, with an average of 8.5 days.

It is during the pupal period only that the sexes can be distinguished. Chapman<sup>9</sup> gives a good illustration of the differences in the terminal segment of the abdomen. On the female there is a pair of appendages in addition to the regular terminal cerci, while the terminal segment of the male appears to have only a disklike depression on this segment.

#### PARASITES

The flour beetles seem to be comparatively free from parasitic enemies. Two mites, *Acarophenax tribolii* Newstead and Duval and *Pediculoides ventricosus* Newport, and a bethylid, *Rhabdepyris zeae* Waterston, have been recorded as attacking *Tribolium*, but none seem to be very effective in controlling these pests.

<sup>9</sup> Chapman, R. N. Op. cit.

## SUMMARY

The flour beetles *Tribolium confusum* Duv. and *T. ferrugineum* Fab. are among the more serious pests of flour and other cereal products. They attack practically any kind of flour, meal, breakfast food, or cracked grain, many kinds of spices, and various nuts, chocolate, and raisins. They are also serious pests in insect collections.

The size of the eyes on the ventral surface of the head is the best character for distinguishing the two species.

The winter is passed in the adult state in the central and northern parts of the United States. Adults of both species may live two years or even longer.

The oviposition period of *T. confusum* may last as long as 14 months, the average being about 9 months. That of *T. ferrugineum* is slightly less. A female of either species usually lays 400 to 500 eggs during this time; in some cases nearly 1,000 eggs have been laid by a single female.

The average incubation period at 27° C. is 6.8 days for *T. confusum* and 5.5 days for *T. ferrugineum*.

The number of larval instars ranges from 6 to 11, with an average of 7 or 8. The larval period at 27° C. ranges from 27 to 90 days according to the food. It is slightly longer for *T. confusum* than for *T. ferrugineum*. Whole-wheat flour, middlings, bran, corn meal, oatmeal, and white flour, arranged in the order of their acceptability, are the foods used in the experiments. Measurements of the head capsule in larvae of *T. confusum* are given.

The pupal period at 27° C. averages 8.2 days for *T. confusum* and 7.1 days for *T. ferrugineum*. Lower temperatures lengthened all stages considerably.

Enemies of the flour beetles include two mites and a bethylid.

# HYPOSOTER DISPARIS VIERECK, AN INTRODUCED ICHNEUMONID PARASITE OF THE GIPSY MOTH<sup>1</sup>

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## INTRODUCTION

*Hyposoter disparis* Viereck, a parasite of the larva of the gipsy moth (*Porthetria dispar* L.) in Europe, was introduced into the United States by the Bureau of Entomology in the course of the early importations of various parasites of that pest made more than 20 years ago. It has become definitely established in part of the area in the New England States which is infested with *P. dispar*, but has remained of little value as a control factor. However, it seems advisable to define as clearly as possible the apparent status of *H. disparis* as a parasite of the gipsy moth, and to present the information concerning its biology and behavior that has been accumulated at the gipsy moth laboratory at Melrose Highlands, Mass.

## SYSTEMATIC POSITION AND REVIEW OF LITERATURE

*Hyposoter disparis* was first described by Viereck in 1911 (9)<sup>3</sup> following its introduction into Massachusetts and after attempts to identify it as a previously described European species had proved unsuccessful. It belongs in the tribe Campoplegini, subfamily Ophioninae, family Ichneumonidae, and order Hymenoptera.

In 1912 Howard and Fiske (3, p. 191) published a few notes on the life cycle of this parasite under the name *Limnerium disparis*; and in 1929 Burgess and Crossman (1, p. 49-52) summarized very briefly the data available at that time on distribution, life history, and introduction into the United States. Otherwise the species has received only incidental mention as a parasite of the gipsy moth.

## DISTRIBUTION IN EUROPE

The original description of *Hyposoter disparis* did not record the type locality more definitely than to indicate that it was European. According to notes at the gipsy moth laboratory, the type specimens appear to have come from Kiev, Russia. In addition, the species has been received from various localities in Austria, Czechoslovakia, Poland, Hungary, Yugoslavia, Bulgaria, and Italy. It appears to be most abundant in south-central Europe.

<sup>1</sup> Received for publication June 18, 1932; issued March, 1933.

<sup>2</sup> The writers are indebted to C. W. Collins, in charge of the gipsy-moth and brown-tail moth investigations of the Division of Forest Insects, Bureau of Entomology, for advice and criticism in the course of this study; to various members of the staff of the gipsy moth laboratory for the accumulation of data concerning the field collections; and particularly to R. Wooldridge, also of the gipsy moth laboratory, for considerable information regarding the habits of the parasite. They are further indebted to R. A. Cushman, of the taxonomic unit of the Bureau of Entomology, for the identification of some of the hyperparasites recorded from *Hyposoter disparis*.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 346.

## IMPORTATION AND COLONIZATION IN THE UNITED STATES

The first cocoons of *Hyposoter disparis*, 20 in number, were received from Russia and Austria in 1907. In succeeding years attempts were made to obtain the species in larger numbers, but it was not until 1911 that cocoons were received in sufficient abundance to provide material for colonization. In that year about 125,000 cocoons of the parasite were collected by agents of the Bureau of Entomology in a gipsy-moth infestation at Gioja Tauro, Italy, and sent to the Melrose Highlands (Mass.) laboratory. Owing to severe hyperparasitism and to considerable mortality during the period of hibernation, from this enormous number of cocoons only about 12,500 adults of *H. disparis* were obtained for colonization the following spring. These adults were liberated in four localities in eastern Massachusetts and at one point in southeastern New Hampshire. Approximately 4,700 were released in North Saugus, Mass.; 3,500 in Andover, Mass.; 2,300 in Boxford, Mass.; and 1,000 each in Wellesley, Mass., and Pelham, N. H.

In 1912 another large shipment, consisting of 171,000 cocoons, was received from Gioja Tauro; but extremely heavy hyperparasitism combined with severe mortality due to the drying out of the cocoons during the winter resulted in an almost total loss of this material, and no liberations could be made in 1913.

Importations of parasites of the gipsy moth ceased with the season of 1912 and were not resumed until 1921. Beginning with 1924 and including 1931, small numbers of *Hyposoter disparis* were received each year incidentally with shipments of other parasites of the gipsy moth from central Europe; but the total for this period was only approximately 5,000 cocoons, of which 3,000 were obtained in 1928 and 1,100 in 1929. Nearly all were from Hungary and Yugoslavia. Since 1912 it has been possible to liberate only three small colonies: One, consisting of 500 adults, in Barnstable, Mass., on Cape Cod, in 1929; another, of 1,862 adults, at Attleboro, in southeastern Massachusetts, in 1930; and a third, of only about 200 adults, in Dighton, Mass., in 1931. The second colony consisted in part of material obtained in reproduction experiments at the Melrose Highlands laboratory.

## RECOVERIES FROM FIELD COLONIES AND DISTRIBUTION IN NEW ENGLAND

Collections of gipsy-moth larvae were made at all five of the 1912 colony sites during the same season in which the liberations were made and *Hyposoter disparis* was recovered from each point. Since 1912 the intensity of the gipsy-moth infestation at these five localities has fluctuated greatly, and in some years collections for the recovery of *Hyposoter* could not be made at all of them. However, the collections that have been obtained have shown that the parasite has persisted at three of the colony sites—namely, Andover, Saugus, and Boxford, Mass.—and possibly at a fourth, Pelham, N. H., and that it has dispersed to some extent, although it has at no time been a parasite of much importance at any of the localities from which collections were secured. It has not been recovered at any of the points at which it was released in 1929, 1930, and 1931.

The present known distribution of *Hyposoter disparis* in New England, as indicated by the recovery records at the gipsy moth laboratory,



appears to be confined to a small area in northeastern Massachusetts and southeastern New Hampshire. (Fig. 1.) The towns in this area from which *Hyposoter* has been recovered are Andover, Boxford, Saugus, Beverly, and Ipswich, Mass.; and Pelham, Derry, Exeter, and Newton, N. H.

#### DESCRIPTION OF THE ADULT

The female of *Hyposoter disparis* (fig. 2) may be described as follows:

Average length 6 mm. Head strongly transverse; face granularly punctate, coarsely so medially; clypeus delicately granular; antennae much shorter than

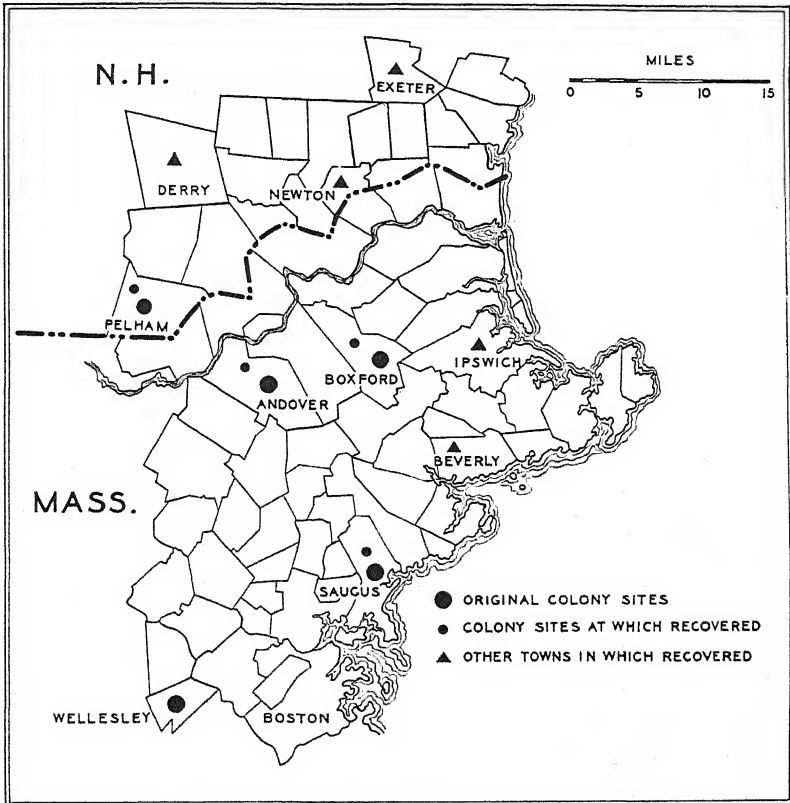


FIGURE 1.—Map showing present known distribution of *Hyposoter disparis*

the body, 32 to 34 segmented; ocell-ocular line longer than diameter of an ocellus. Thorax short and stout, mostly closely granular, the scutellum more weakly so than the mesoscutum; propodeum not extending beyond base of hind coxae, incompletely areolated, areola and petiolar area confluent, the latter a little broadly impressed down the middle and more or less transversely rugulose; anterior wing with a small petiolate areolet. Abdomen somewhat thickened and a little compressed posteriorly, mostly delicately alutaceous; petiole smooth at base; second tergite broadening strongly behind, with the spiracles at about the middle; ovipositor sheaths short, scarcely as long as apical truncature of abdomen. Black; mandibles, except at tips, and the palpi, pale yellow; scape and pedicel of antennae yellowish brown; wings hyaline; legs, including all coxae, testaceous; posterior tibiae slightly dusky at base and broadly black at apex; second abdominal tergite broadly reddish testaceous posteriorly.

In essential characters the male is similar to the female.

*Hyposoter disparis* is easily confused with an unidentified species of the same genus which is occasionally reared as a parasite of gipsy-moth larvae in Europe. The latter differs, however, in having the antennae 28 to 30 segmented; in the ocell-ocular line being slightly shorter than the diameter of an ocellus; in the less erect areolet of the anterior wing, with the second recurrent joining the cubitus very

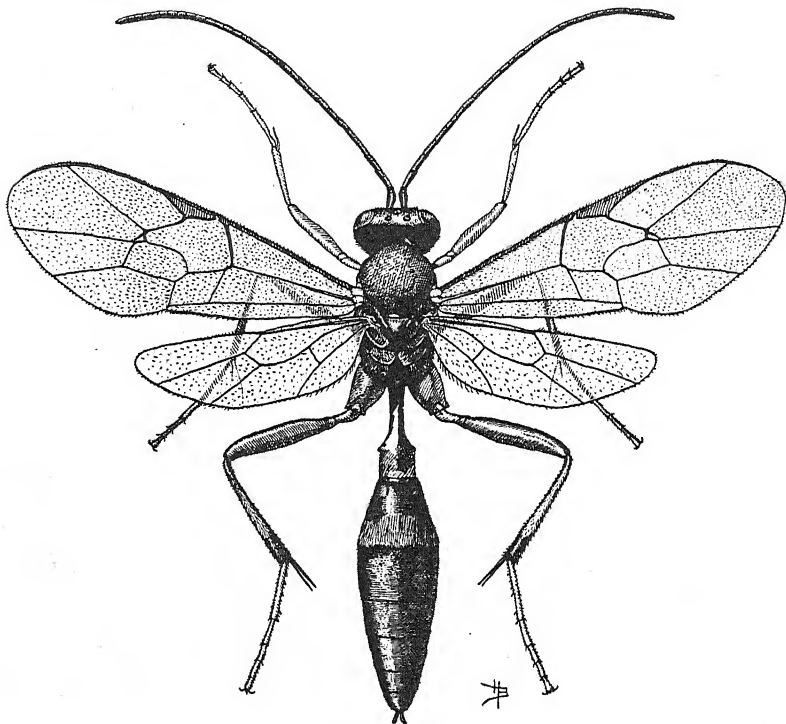


FIGURE 2.—*Hyposoter disparis*, female.  $\times 12$

near the second intercubitus; and in having the petiolar area wider and uniformly closely granular and opaque.

### LIFE HISTORY AND HABITS

#### HIBERNATION

*Hyposoter disparis* hibernates as an adult within its cocoon, the posterior end of the body remaining immersed in the moist meconial discharge. If the meconium dries the parasite dies in the cocoon. Consequently it is important that the cocoons be held sufficiently moist to maintain the semiliquid condition of the meconial discharge. In the field they remain on the surface of the ground throughout the winter, although usually covered to some extent by fallen leaves. At the laboratory, hibernation of *H. disparis* has not been very successful until recently, apparently because the proper moisture conditions have not been supplied. Attempts to simulate field conditions by placing the cocoons on sod or soil and covering them with dried

leaves failed, for the emergence of adults from lots of cocoons held in this manner has been very poor.

In some of the more recent attempts to carry the parasite through the hibernation period, glass cylinders with bottoms of plaster of Paris have been employed. These cylinders are about 3 inches in diameter and range from 3 to 5 inches in height, while the plaster of Paris floors are about half an inch thick. Emergence exceeding 50 per cent was sometimes secured by spreading the cocoons in a thin layer on the floor of such a container and placing the container in a pit partly filled with soil, the cylinder itself being set down about an inch into the soil. The top of each cylinder was covered with cheesecloth and the entire pit sheltered from the rain. However, more or less loss resulted from the molding of the cocoons, and in some cases the mortality was high. Apparently this was due to an excessive amount of moisture drawn up through the plaster of Paris. To obviate this the plaster of Paris was covered with half an inch of sawdust before the cocoons were placed in the container. The results have been highly satisfactory. This method was employed in the hibernation of 2,471 cocoons in 1929, and of these 2,023, or about 82 per cent, produced adults the following spring, which was much the best emergence that had been obtained. Neither the glass cylinder nor the plaster of Paris is essential, for equally good emergence was secured by substituting galvanized-metal cylinders having bottoms of fine wire screening upon which an inch of sawdust had been placed.

#### EMERGENCE AND MATING

Adults of *Hyposoter disparis* appear to emerge at about the time the eggs of the gypsy moth begin to hatch. In the case of the material hibernated in outdoor cages at the Melrose Highlands laboratory, emergence has usually extended from about April 25 to May 10.

It has been found rather difficult to obtain satisfactory mating in confinement. Most of the earlier attempts were altogether unsuccessful. More recently, however, some degree of success has been attained by employing a cloth-covered cage about 13 inches long, 9 inches wide, and 9 inches high provided with a sliding glass front through which the parasite could be observed and which permitted the easy removal of mating pairs. Best results were secured when not more than 20 to 25 males and 8 to 10 females were used at one time in a cage of this type. Furthermore, it was found that temperatures of 65° F. or higher were required to stimulate the parasites to the necessary activity, and that sunlight was essential. Occasionally females a week old mated in such cages, but the most satisfactory mating was obtained when freshly-emerged females were used with males that were about 3 or 4 days old.

#### OVIPOSITION

Females of *Hyposoter disparis* oviposit most readily in first and second instar larvae of the gypsy moth. Apparently larvae of the first instar are preferred, although in reproduction experiments at the gypsy moth laboratory larvae of the second instar have usually been employed owing to the greater ease of handling and the somewhat lower mortality in the rearing trays.

The ovipositing parasites prefer to attack moving caterpillars. Usually, if a female comes upon a larva that is not moving, she slowly encircles it, vibrating her wings and antennae rapidly and occasionally prodding the caterpillar with her ovipositor. Generally the larva soon begins to crawl away, sometimes very rapidly, with the parasite in pursuit. After several additional quick thrusts she finally inserts her ovipositor and deposits an egg, this act requiring about a second. Owing to the habit of the parasites of attacking the host larva in this manner, the eggs are usually deposited in the posterior part of the body. On dissection eggs have frequently been found even in the rectum of the caterpillar, although normally they are placed in the body cavity. Several eggs may be deposited in one host, even in the field, but only one parasite can mature.

#### POTENTIAL REPRODUCTIVE CAPACITY

An attempt was made to secure some information concerning the number of eggs that may be deposited by a single female of *Hyposoter disparis*. Ten unmated females that had had no opportunity to oviposit were isolated each in a glass vial 8 inches long and 2 inches in diameter, and early each day six first-instar larvae of the gipsy moth were introduced into each vial. Late in the afternoon these



FIGURE 3.—Egg of *Hyposoter disparis*.  $\times 120$

larvae were removed and dissected and the eggs deposited by each parasite counted. The total number of eggs deposited by the individual females ranged from 182 to 1,228, with an average of 561 for the 10 parasites. The first death occurred after 12 days and the last after 54 days. In most cases the female continued to oviposit until the day of her death, but the specimen that lived 54 days deposited no eggs after the 34th day, although repeatedly observed to insert the ovipositor in larvae offered after that time. In many cases 50 or more eggs were deposited by a single female on one day, and in one instance as many as 84 were deposited.

#### EGG

The egg of *Hyposoter disparis* (fig. 3) at the time of deposition measures 0.40 to 0.45 mm in length and 0.11 to 0.14 mm in extreme width. It is slightly kidney shaped, smooth, and pearly white. After deposition in the host, the egg gradually increases in size and before hatching attains a length of 0.7 to 0.8 mm and a width of 0.25 to 0.28 mm. Normally the egg stage covers a period of about 7 days, although under unusually low spring temperature this may be extended to 10 days.

#### LARVA

Only three larval instars seem to have been observed in the case of related Campoplegini that have been studied in some detail. Timberlake (7) found only three in *Eulimnerium validum* (Cresson); likewise Tothill (8) in his study of *Hyposoter pilosulus* (Provancher) and Thompson and Parker (6) in their work on *Eulimnerium crassifemur* (Thomson) mention but three instars. In the course of the present study hundreds of dissections have been made of gipsy-moth larvae which were parasitized by *Hyposoter disparis* at the laboratory, and detailed examinations of the numerous series of *Hyposoter* larvae

obtained in this way have enabled the writers to distinguish clearly five larval instars in this species.

The first-instar larva is elongate, more or less cylindrical, and smooth, with a strongly sclerotized brown head, and a long caudal appendage, which is a prolongation of the last, or thirteenth, body segment. On hatching, the larva measures about 1.2 mm in length, including the anal appendage, which itself is 0.30 to 0.35 mm long. The mandibles (fig. 4, A) are small, but heavily sclerotized and strongly hooked. Apart from its larger size, the larva of the second instar differs from that of the first principally in its less sharply defined head, differently shaped and less sclerotized mandibles, and the somewhat shorter caudal appendage. The larvae of the third and fourth instars are similar to the second-instar larva, except for the increase in size, and can be distinguished with certainty only by the mandibular differences. In the large quantity of material examined the mandibles in each instar are shown to be remarkably constant in size and form. Identification of the instars was greatly facilitated by the fact that certain larvae, obtained in the course of the numerous dissections, were in the act of molting and accordingly supplied the mandibles of two successive instars.

In the mature larva the mandibles are more heavily sclerotized than in the second, third, or fourth instar; the labial ring and the sclerotic framework in the mouth region are brown in color and conspicuous; the body integument is covered with minute tubercles; the antennal, leg, and wing pads can be distinguished; and there are nine pairs of open spiracles, one pair near the posterior margin of the first thoracic segment and a pair on each of the first eight abdominal segments. The caudal appendage is greatly reduced and resembles a short, thick, evenly tapering spine. In length the mature larva measures 8 to 10 mm.

The time spent in each instar varies considerably, being dependent chiefly on the temperature. From the large number of dissections it was determined that 5 to 10 days are spent in the first instar, 2 to 5 days in the second, 2 to 4 days each in the third and fourth instars, and 1 to 2 days in the fifth, while the parasite is still within the host.

The host is killed several hours before the mature parasitic larva emerges and upon the issuance of the latter is left as a flaccid empty skin, practically the entire contents having been consumed. Almost invariably the gypsy-moth larva is in the fourth instar when it is killed by the parasite.

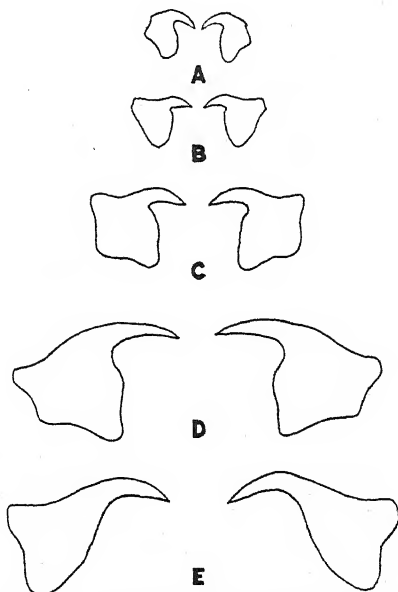


FIGURE 4.—Mandibles of larva of *Hyposoter disparis*: A, of first-instar larva; B, of second-instar larva; C, of third-instar larva; D, of fourth-instar larva; E, of fifth-instar larva. All drawn to same scale

## EFFECT OF TEMPERATURE ON DEVELOPMENT

In reproduction experiments conducted in 1929 the period from deposition of the egg to formation of the cocoon ranged from 20 to 33 days, while in 1930 the corresponding figures were 27 to 41. The temperatures during the experiments in 1929 were distinctly higher than during the experiments in 1930. The readings were obtained from the records of a thermograph in the laboratory yard, and daily mean temperatures were computed by averaging the hourly readings. In 1929 the average of the daily mean temperatures for the period concerned was 63.7° F., while in 1930 it was 57.6°.

No doubt this difference was, at least in large part, responsible for the difference in the length of the developmental periods for the two years.

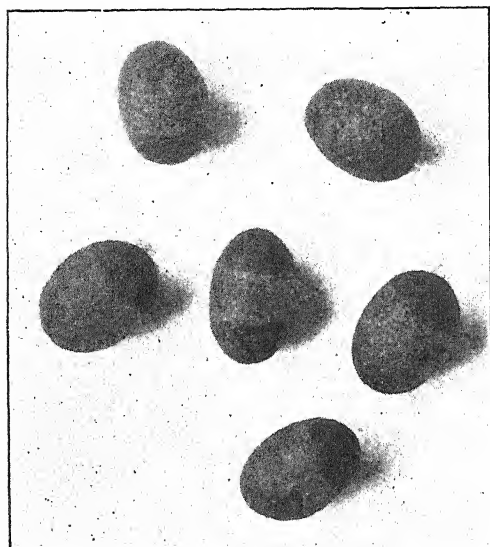


FIGURE 5.—Hibernating cocoons of *Hyposoter disparis*.  $\times 2\frac{1}{2}$

## COCOON

The cocoon of *Hyposoter disparis* is ovoid in shape and measures about 6 to 7 mm in length and 4 to 4.5 mm in extreme transverse diameter. (Fig. 5.) It is dark gray in color, with a broad, pale gray, transverse band around the middle. An outer layer of comparatively loose silk covers the dense, tough, more or less parchimentlike envelope. Normally the cocoons are formed on the underside of leaves or branches beside the dead host larvae.

The attachment to the remains of the host or to the surface of the leaf or branch is so weak, however, that the cocoons invariably, so far as observed, drop to the ground, in most cases apparently within 48 hours after being formed.

## PUPA

In order to determine the time of pupation and the length of the pupal stage, numerous dissections were made of cocoons of known ages. Twenty-four hours after completion of the cocoon the short caudal appendage of the mature larva has been retracted and has become shrunken and dark in color. After four days in the cocoon the larva exhibits a slight constriction at the posterior margin of the thoracic region and the developing eyes are weakly discernible. The meconium is usually cast about six days after formation of the cocoon and actual pupation occurs on the ninth or tenth day. The pupa darkens gradually, until at the end of about five days the head and thorax have become black and the base of the abdominal petiole is beginning to darken. Transformation to the adult occurs usually 20 to 21 days after the cocoon is completed, the pupal stage thus covering about 11 days. Normally the adult stage within the cocoon is at-

tained during the first half of July, although emergence does not occur until the following spring, there being but a single generation annually. Very rarely, however, a male adult may emerge the same season in which the cocoon is formed.

#### HYPERPARASITISM

Because *Hyposoter disparis* has not been abundant in any of the localities in Massachusetts and New Hampshire in which it has been found, and since the cocoons, which drop to the ground soon after they are formed, are difficult to find in the field unless the parasite is present in large numbers, no records have been obtained concerning the extent to which *H. disparis* is attacked by hyperparasites in this country. However, as already mentioned, the cocoons received from Italy in 1911 and 1912 were heavily infested with secondary parasites. The species reared included *Hemiteles areator* Gravenhorst; three unidentified species of the same genus; *Gelis* sp.; *Therioscopus* sp. (?); *Spilocryptus pumilus* Kreichbaumer, var.; *Thysioiurus* sp.; *Theronia atalantae* (Poda); *Itoplectis clavicornis* (Thomson); *I. alternans* (Gravenhorst); *Monodontomerus aereus* Walker; *Monodontomerus* sp.; *Haltichella maculipennis* De Stefani; and *Eurytoma appendigaster* (Swederus). Unfortunately, accurate data concerning the percentage of parasitism by each or all of these species are not available.

#### ECONOMIC IMPORTANCE

As already mentioned, *Hyposoter disparis* has not yet become an important parasite of the gypsy moth in New England. Year after year, since the original liberations in 1912, the species has been obtained from one or more localities in the course of the intensive rearing work that has been conducted by the gypsy moth laboratory in order to determine the distribution and effectiveness of the introduced parasites of the gypsy moth; but usually it has been reared in such small numbers that the recoveries merely served to indicate that the parasite was still present. However, in 1929, 1930, and 1931 cocoons were obtained in somewhat larger numbers and from a gradually increasing number of localities. In several collections the parasitism by *H. disparis* has been 5 to 7 per cent. Moreover, dissections of a collection of 205 gypsy-moth larvae obtained at Boxford, Mass., in 1930 showed parasitism of 28 per cent by this species.

Members of the staff of the gypsy moth laboratory had observed in certain European infestations of the gypsy moth that cocoons of *Hyposoter disparis* occurred most abundantly in dense woodland, and that they could be found only occasionally along the outer margins or on the edges of clearings. A similar condition in New England infestations within the area where *H. disparis* occurs had been suggested by certain observations. Accordingly, since rearings in 1929 had indicated an increase in the abundance of the parasite at Boxford, Mass., in the spring of 1930 an attempt was made to obtain more exact information on this point. The gypsy-moth infestation at this locality had declined sharply, and consequently only small collections of larvae could be obtained. Nevertheless, the data secured are of interest. In the dense woodland 205 gypsy-moth larvae were collected while at the same time 230 were taken along the edges of this

woodland, all larvae of both lots being obtained from an area comprising not more than 3 acres. All were then dissected. In the larvae collected on the outer margin of the woodland the parasitism by *H. disparis* was only 4 per cent, while those larvae obtained in the shaded parts of the area were 28 per cent parasitized. This is of particular interest since certain other hymenopterous parasites of the gipsy moth have repeatedly been observed to parasitize a higher percentage of the host larvae in the more open growth than in the midst of the woodland.

In the many infested areas in Europe where the gipsy moth laboratory has conducted extensive rearing work during the last 10 years, *H. disparis* has never been observed to be a parasite of more than minor importance. Nevertheless, even species in that category may under certain conditions become the determining factor in the decline of an infestation. Moreover, the fact that *H. disparis* occurred in such enormous numbers in a gipsy-moth infestation at Gioja Tauro, Italy, in 1911 and 1912 that 125,000 and 171,000 cocoons, respectively, could be collected in the two years shows that under some conditions the species may become an important parasite of the gipsy moth.

Little information has been obtained concerning other hosts of *Hyposoter disparis*. At the European station of the gipsy moth laboratory at Budapest, Hungary, large numbers of larvae of many species of Lepidoptera have been reared each year since 1926 in order to obtain information regarding possible alternate hosts of various parasites of the gipsy moth in Europe, but *H. disparis* has never been reared from any of the numerous species collected. In experiments at the Melrose Highlands laboratory the parasite successfully reproduced on larvae of the brown-tail moth (*Nygmia phaeorrhoea* Donovan), but this species does not appear to be so suitable a host as the gipsy moth.

Even the gipsy moth does not seem to be an altogether satisfactory host. Although a single female is capable of depositing a large number of eggs, this potential effectiveness is offset to a considerable extent by failure of eggs to hatch after being deposited in the host or by the early death of the parasitic larva. Large numbers of dissections have shown many parasitized larvae of the gipsy moth to contain dead eggs, and occasionally a dead larva of *H. disparis* surrounded by phagocytes.

#### PHAGOCYtic REACTION OF THE HOST

Numerous investigators have observed that internal parasites are sometimes surrounded by phagocytes of the host and most of them have assumed that the parasites had been killed as a result of this phagocytosis, which was considered a defensive measure of the host against the invasion of the parasite. However, Cuenot (2) observed that some parasites cause a phagocytic reaction to be set up by the host while others do not; and Pantel (4, p. 158-160) concluded that normal healthy parasites do not induce this response, but that the phagocytes accumulate about dead or weakened parasites as they do about inert objects that may find their way into the body of the host. Timberlake (7), in his study of the campoplegine *Eulimnerium validum* (Cress.), observed that phagocytosis occurred very commonly when eggs of this parasite were deposited in certain hosts, particularly the



brown-tail moth (*Nygmia phaeorrhoea*) and the eastern tent caterpillar (*Malacosoma americana* Fab.), but not at all when oviposition had occurred in the fall webworm (*Hyphantria cunea* Drury), the normal host of the Eulimnerium. He concluded that normal, or adapted, parasites do not arouse this phagocytic response, which he considered a defensive reaction on the part of the host, while those parasites that are unadapted commonly do. But more recently Thompson (5), after wide experience involving observations on the development of many different types of internal parasites, has stated that in his opinion living parasitic larvae occurring free within the body of the host are never attacked by phagocytes, and that the adaptation of the parasite does not consist in the development of substances or structures to give protection against the attacks of phagocytes.

In their study of *Hyposoter disparis* the writers have commonly found eggs of this parasite surrounded by phagocytes of the host. Much more rarely first-instar larvae have been found in this condition. In the course of the experiment, already mentioned, in which an attempt was made to determine the potential reproductive capacity of *Hyposoter disparis*, 3,427 eggs of this parasite were dissected from the gypsy-moth larvae that had been subjected to attack. It is worth noting that of these, 356, or more than 10 per cent, were surrounded by phagocytes within eight hours after oviposition. Furthermore, the dissections of host larvae made with the object of following the development of the *Hyposoter* larva showed that 13 per cent of the eggs and larvae found were dead and inclosed by phagocytes; and in dissections of several hundred field-collected larvae of *Porthetria dispar*, 42, or about 34 per cent, of the 123 eggs and larvae of *H. disparis* found, were noted as encysted.

The fact that the eggs and larvae of *Hyposoter disparis* so commonly fail to develop in caterpillars of the gypsy moth and become surrounded by phagocytes has suggested that the adaptation of this parasite to *Porthetria dispar* is not complete, especially since no similar condition has been observed with any of the other parasites introduced as agents for the control of this pest. Apparently the egg or larva of the parasite dies before encystment occurs, perhaps because the medium in which the parasite finds itself is not altogether suitable for its development.

#### SUMMARY

*Hyposoter disparis* Viereck is a parasite of the larva of the gypsy moth in Europe. It is known to occur in Russia, Poland, Austria, Czechoslovakia, Hungary, Yugoslavia, and Italy, being apparently most abundant in the south-central part of the Continent.

It was first introduced into the United States in 1907 but it was not colonized until 1912. Five colonies comprising 12,500 adults were released in 1912, and the parasite immediately became established. It has dispersed only slightly, however, its present distribution in New England being restricted to a small area in northeastern Massachusetts and southeastern New Hampshire.

The adult parasite is about 6 mm long, with a short, compact thorax, an elongate abdomen, and, in the female, a short, inconspicuous ovipositor. In color it is black with a broad reddish-yellow band on the second abdominal segment, reddish-yellow legs, and hyaline wings.

The species hibernates as an adult in the cocoon, and emergence occurs normally late in April or early in May. Mating under conditions of confinement was best obtained by the use of a small cloth cage held out-of-doors in direct sunlight. Temperatures of 65° F., or higher, were essential. The parasite oviposits most readily in active gipsy-moth larvae of the first and second instars. As many as 1,228 eggs were deposited by one female, and the average for 10 individuals was 561.

The egg is slightly kidney-shaped, smooth, and pearly white; it is about 0.4 mm long when first deposited, but increases in size before hatching. There are five larval instars. The first-instar larva is elongate, cylindrical, with a strongly sclerotized brown head and a long caudal appendage. When mature, the larva measures 8 to 10 mm in length, the mandibles and the framework of the mouth region are heavily sclerotized, there are nine pairs of open spiracles, and the caudal appendage has become greatly reduced. The period from egg deposition to formation of the cocoon ranges from about 20 to about 40 days, and depends in large part on the temperature. The cocoon is ovoid, about 6 to 7 mm long, dark gray in color, with a broad, complete, transverse pale-gray band at the middle. Pupation occurs 9 or 10 days after cocoon formation, and transformation to the adult 10 or 11 days later. There is but one generation annually.

*Hyposoter disparis* has, on rare occasions, been observed to be a very abundant parasite of the gipsy moth in Europe, but it has not yet become an important control agent of that pest in New England. Apparently the parasitism is heavier in dense woodland than in open growth or on the outer edges of wooded areas.

The potential effectiveness of the species as a parasite of the gipsy moth is offset to a considerable extent by failure of eggs to hatch or by early death of the parasitic larvae. From 10 to more than 30 per cent of the eggs and young larvae of the parasite dissected from host caterpillars that had been attacked in the laboratory and in the field were found to be dead and surrounded by phagocytes of the host. This suggests incomplete adaptation of the parasite to this host.

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# CURVED-LINE RELATIONSHIPS BETWEEN CERTAIN SHORT-PERIOD EGG YIELDS AND ANNUAL EGG PRODUCTION IN SINGLE-COMB WHITE LEGHORN FOWLS<sup>1</sup>

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## INTRODUCTION

Scientific investigators and practical poultry producers have long since agreed that capacity to produce increased numbers of eggs is, at least to some considerable extent, inherited. It is obvious that the successful poultry farmer must (1) secure as high an average egg yield per year per bird as is possible without impairing the health of the birds, and (2) employ certain principles of selection and breeding to accomplish this. Poultrymen recognize differences between the individual birds in their flocks in egg-producing capacity. They realize that criteria of selection must be used in establishing breeding flocks from which future layers are to be produced. The trap nest has been employed as a mechanical means of securing a measure of egg production. The exact record of daily egg yield for each individual for the entire pullet year of production has proved to be a useful and reliable criterion in selecting breeding stock. The trap-nest method, however, is so laborious and expensive that its general use is prohibitive, therefore materially reducing the number of accurately recorded birds from which to choose breeders.

The purpose of the present study, which is based on correlations previously reported between winter-season egg yield and yearly total egg production, was to investigate the nature and type of such correlation, to make a similar correlation with another short period, and to determine the possibilities of drawing up a workable, practical breeder-selection program.

## REVIEW OF LITERATURE

A survey of the literature indicates that several workers have noted that some significance attaches to the egg-yield record made during the winter season; that is, during the first three or four months of the first, or pullet, laying year.

Ball and Alder (*l. p. 53-54*)<sup>2</sup> in 1917 made some interesting observations:

Environmental factors influence the records of the pullet year more than that of later years and influence flocks making low records more than those making high ones. \* \* \* Winter egg production of flocks is more variable than annual production. This variation seems to be closely correlated with environmental factors. \* \* \* The correlation between "winter" production and annual production was subject to wide and irregular fluctuations in the different flocks, without reference to the height of production. \* \* \* The average correlation was 58 per cent. The ("winter") period as used does not seem accurately to represent a biological entity, but is made up of the end of one period and the beginning of another.

<sup>1</sup> Received for publication Apr. 26, 1932; issued March, 1933. Journal Series Paper of the New Jersey Agricultural Experiment Station.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 353.

This contribution from the Utah station was part of a rather extensive series of studies on the economic significance of various phases of egg production over a period of years. This work did much to impress upon poultry breeders the differences between individual fowls.

Harris, Blakeslee, and Kirkpatrick (2) in 1918 reported that in the Single-Comb White Leghorn fowl, under reasonably uniform conditions of breeding and management (they used Storrs egg-laying-contest records), there was a significant positive correlation between the number of eggs laid by a bird in any month of the year and the number of eggs laid during the whole year. They further observed that there was a more intimate correlation between the egg production of the autumn and winter months at the beginning and end of the contest year than between the egg production of these months and that of the spring and summer months.

Harris, Kirkpatrick, Blakeslee, Warner, and Card (3) in 1921 again presented evidence to the effect that annual egg production for the pullet year might "with a high degree of accuracy" be predicted when the known yield for one, two, or three months was available. They suggested that full-year trap nesting, admittedly expensive in operation, might not always be necessary.

Harris and Lewis (4) in 1922, using New Jersey egg-laying-contest data, similarly indicated a sufficient degree of association between certain short-period trap-nest records and annual egg-production records to warrant their use for prediction purposes.

Hervey (7, 8) in 1923 and 1924 continued studies of the correlation between short-period egg yield and annual total yield and described such relationships in a way that would be useful to practical poultry breeders. Hervey (7) reported a coefficient of correlation of  $+0.7752 \pm 0.0132$  between winter egg yield and annual total, on the basis of which, using a straight-line equation, he offered a regression equation for prediction of annual egg production when the winter record was known, as follows:  $Y \text{ (annual)} = 1.411X + 116.8$ .

Hervey (7, p. 6) stated:

Precedent for the use of the straight line has been set by Harris in a series of reports with other authors, \* \* \* not with the idea that regression is always linear when production is considered, but in the absence of a large amount of data to prove the contrary its use seems justifiable.

Hays, Sanborn, and James (6) in 1924 reported on breeding work in the Rhode Island Red in which they indicated a coefficient of correlation of  $+0.6214 \pm 0.0142$  between winter yield and annual total and stated that the winter record was of significant value in the selection of pullet breeders. Hays (5) later presented evidence of a highly significant degree of association between winter and annual egg production.

These, and other workers, have paved the way for drawing up a practical breeder-selection program based on the use of short-period trap-nest records. So far as the present author has been able to determine, previous workers have all used a straight line to express relationship between the short-period trap-nest record (in all cases up to this time this short period has been either a month, or the winter season, except in the work of Hervey (7)). Practical poultrymen have at times expressed dissatisfaction with the use of such straight-line equations, feeling that the results were not sufficiently accurate to be

used in substitution for full-year records. Some of these points of view raised questions which a review of the previous work did not seem fully to answer. Several poultrymen have pointed out that such studies as these would possibly have increased interest and value if the information thus obtained could be applied on poultry-breeding plants where whole flocks were placed under trap nest on set dates and where it was not practicable to get individual records from the first egg. Hays et al. (6) of course had reported on a certain strain of the Rhode Island Red. They also used data from the first egg.

#### MATERIALS AND METHODS

During the two years following October 1, 1929, several thousand pullets were accurately trap nested and their respective egg yields definitely recorded at one or another of the three official egg-laying contests operated under the management of the Department of Poultry Husbandry, New Jersey Agricultural Experiment Station, and located at Paterson, Flemington, and Vineland, N. J. Included in this number were several thousand Single-Comb White Leghorns, a variety used extensively in the commercial table-egg industry. Upon examination of the available trap-nest records it was found that there were complete full-year (pullet-year) records at hand for 3,937 individuals, each one of which had lived throughout the first contest year (October 1 forward for 51 weeks). These individuals, which have been used in this study, were found distributed through 375 flocks. The flocks in the main represented as many different lines of breeding, since each flock in the egg-laying contest consisted of 16 birds sent by the poultry breeder. The sample is considered representative of the Single-Comb White Leghorn variety in the Eastern States. The various probable errors computed during the work tend to give evidence that this statement is correct.

It should be recalled that the pullets sent to egg-laying contests are in the great majority of cases so selected that they are in a stage of maturity which will bring them into laying condition at a time very near to the opening of the contest year. Thus it is safe to assume that the records here used were gathered from birds so hatched and reared as to bring them into production on approximately October 1. It is this definite group of birds on which this study has been made, and, therefore, it is for such type of birds that the results would naturally apply.

The egg yield during the first 120 days of the egg-laying-contest year—that is, from October 1 through January 28—was considered as the winter period, and the egg yield during this period (although the limits of the period were arbitrarily set) was used as a measurement of the pullet's capacity to produce eggs during the early weeks of the first production year. It was assumed that (1) pullets reach the date of first egg, or sexual maturity, under average conditions, physically strong and vigorous; (2) pullets are likely to produce eggs chiefly as a result of inherited capacity during the earlier weeks of the production year; and (3) production will probably be less affected by environmental factors during this winter period than during the full-year period. If these premises are correct, the record of egg yield during the winter period takes on an unusual significance and gives promise of being an economically useful criterion of selection. Evidence already submitted substantiates this view.

The egg yield during the last 30 days of the contest year—that is, during the period between August 25 and September 23, inclusive—was adopted as a short period at the end of the pullet year of production which might give a practical picture of a tendency to maintain egg production well through the summer-fall season. In the industry it is generally recognized that pullets which are able to keep up egg yield well through the hot summer months and into September are usually among the more prolific egg producers of the flock. Because of market-egg prices during this summer-fall period and because of the general labor situation on the average farm at that time egg yield during August and September is of considerable importance. The dates are arbitrarily selected.

Prof. C. S. Platt of the New Jersey station suggested to the author that the addition of the two short-period records just referred to might become a still more useful criterion of selection than either used separately, and that the essential data obtained might not be impracticable for general use. For purposes of brevity this combined period is called the “120+30” record.

The following series of correlations are apparently essential:

- (1) Between winter egg yield ( $X_1$ ) and annual total egg production ( $Y$ )
- (2) Between winter egg yield ( $X_1$ ) and the last 30-day yield ( $X_2$ )
- (3) Between last 30-day yield ( $X_2$ ) and annual total egg production ( $Y$ )
- (4) Between annual egg production ( $Y$ ) and winter egg yield ( $X_1$ ), with the last 30-day egg yield ( $X_2$ ) held constant.
- (5) Between the “120+30” yield ( $X$ ) and annual total egg production ( $Y$ ).

After the nature of these several correlations was determined, a series of regression equations for prediction purposes was derived. These are given later in this paper.

It was planned to formulate, if possible, a practicable selection program for the use of poultry breeders, embodying the use of short-period trap-nest records and their relationship to the full-year trap-nest record.

#### ANALYSIS OF EGG-PRODUCTION RECORDS

Methods of statistical technique were applied to the available 3,937 pullet-year egg-production records in an effort to obtain the necessary facts and figures for the interpretation of these records. The results are presented as problems 1, 2, and 3, respectively. In all problems the value of  $N=3,937$  cases.

Problem 1. Correlation between winter egg yield and total annual egg production,  $X_1$  and  $Y$ , respectively:

	For $X_1$	For $Y$
(1) Mean, with its probable error.....	$56.6 \pm 0.2474$	$202.96 \pm 0.8241$
(2) Standard deviation, with its probable error.....	$21.1 \pm 0.1607$	$51.71 \pm 0.3928$
(3) $r_{xy}$ .....	$+0.6352 \pm 0.0064$	
(4) $\eta_{yx}$ .....	$+0.6641 \pm 0.0033$	
(5) Blakeman's test for linearity (short) showed $12.1538 > 4.047$ , and therefore a curvilinear relationship was indicated between $X_1$ and $Y$ .		
(6) Regression equation (straight line, i. e., $Y=a+bX_1$ ) was found to be, with its standard error of estimate, as follows: $Y=1.553 X + 115.032 \pm 30.8435$ .		
(7) Curvilinear regression equation ( $\log Y=a+b \log X_1$ ) was found to be $\log Y=1.24851+0.6143 \log X_1$ .		
(8) Class interval for $X_1=10$ eggs, and for $Y=25$ eggs.		

Problem 2. Correlation between winter egg yield and total annual egg production,  $X_1$  and  $Y$ , respectively, with last 30-day egg yield ( $X_2$ ) held constant:



- For  $X_2$
- (1) Mean, with its probable error.....  $19.4 \pm 0.0656$
  - (2) Standard deviation, with its probable error.....  $6.1 \pm 0.1473$
  - (3)  $r_{12,3}$ .....  $+0.7487$ , when 1 refers to annual egg production, 2 to winter yield, and 3 to last 30-day yield.
  - (4)  $r_{12}$ .....  $+0.6352 \pm 0.0064$   
 $r_{13}$ .....  $+0.6024 \pm 0.0069$   
 $r_{23}$ .....  $+0.0610 \pm 0.0681$
  - (5) Partial regression equation ( $Y = b_{12,3} X_1 + b_{13,2} X_2 + C_1$ ) was found to be  $Y = 1.4692 X_1 + 4.7905 X_2 + 26.9437 \pm 18.4063$ .

Problem 3. Correlation between the "120+30" egg yield and total annual egg production,  $X$  and  $Y$ , respectively:

- For  $X$
- (1) Mean, with its probable error.....  $61.0584 \pm 0.4584$
  - (2) Standard deviation, with its probable error.....  $42.6404 \pm 0.3241$
  - (3)  $r_{xy}$ .....  $+0.6711 \pm 0.0088$
  - (4)  $\eta_{yx}$ .....  $+0.7150 \pm 0.0078$
  - (5) Blakeman's test for linearity (short) showed  $8.84084 > 4.047$ , and therefore a curvilinear relationship was indicated between  $X$  and  $Y$ .

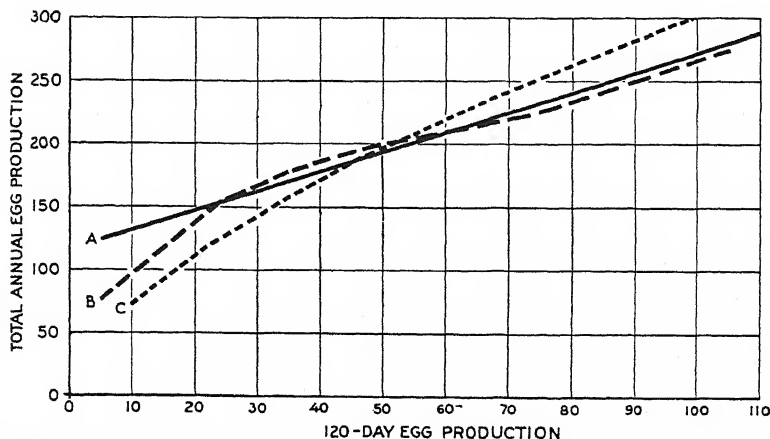


FIGURE 1.—Correlation between egg yield during first 120 days (as  $X$ ) and total annual egg production (as  $Y$ ): A, Regression of  $Y$  on  $X$  (straight line,  $Y = a + bX$ ); B, line connecting means of columns; C, regression of  $Y$  on  $X$  (curved line,  $\log Y = a + b \log X$ )

- (6) Regression equation (straight line, i. e.,  $Y = a + bX$ ) was found to be, with its standard error of estimate, as follows:  $Y = 0.8138 X + 153.2667 \pm 28.4184$ .
- (7) Curvilinear regression equation ( $\log Y = a + b \log X$ ) was found to be  $\log Y = 1.2324 + 0.64208 \log X$ .
- (8) Class interval for  $X = 10$  eggs.

The results here indicated are of considerable interest, especially in that (1) the size of  $N$  (3,937 cases) is very much greater than has been the case in earlier work and is sufficiently great to merit attention as a fair cross section of the possible universe, (2) a curvilinear relationship is indicated as existing between winter egg yield and the annual egg-production total, whereas in earlier reports only linear relationship has been dealt with, (3) a distinctly curvilinear relationship is shown between the "120+30" egg yield and the annual egg-production total, and (4) that an even greater and more significant correlation exists between the "120+30" egg yield and the annual yield than between winter yield and annual production.

<sup>3</sup> All values were carried to the fourth decimal place for use in computing.

Figures 1 and 2 show certain of these data.

The poultry population of an egg-laying contest is closely akin in type to that which would be found on the poultry-breeding farms from which the contest-flock birds have come. The poultry breeder selects certain of his maturing pullets to be trap nested, part of them are sent to a contest and part are trap nested at home. The 3,937 individuals used in this investigation are considered, therefore, as representing a fair cross section of Single-Comb White Leghorn pullet layer stock. Figure 3 shows the distribution data of the 3,937 individuals as to egg yields during the several short-period intervals and during the full-year period.

In each instance the points located represent the frequencies at the respective midpoints of the chosen intervals. Frequencies have been plotted on the ordinate, and egg yields along the abscissa.

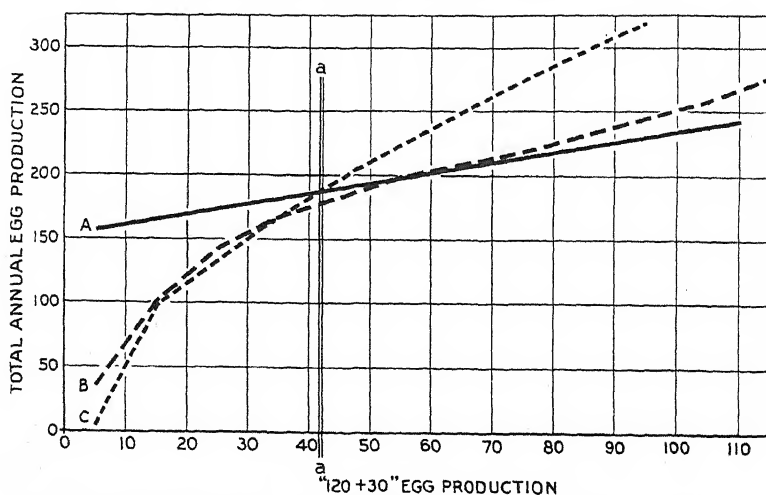


FIGURE 2.—Correlation between egg yield during first 120 days plus last 30 days (as  $X$ ) and the total annual egg production (as  $Y$ ): A, Regression of  $Y$  on  $X$  (straight line,  $Y=a+bX$ ); B, line connecting means of columns; C, regression of  $Y$  on  $X$  (curved line,  $\log Y=a+b \log X$ ); aa, division line (see text)

## DISCUSSION OF EGG PRODUCTION RECORDS

Poultry breeders are apparently using egg-laying contests in a serious attempt to identify future breeders of increased egg-production capacity. This is indicated in the distribution of cases as to annual egg production. A study of Figure 3 shows that in these 3,937 cases 84 per cent of the individuals produced between 150 and 274 eggs, inclusive, 13 per cent produced between 0 and 149 eggs, inclusive, and only 3 per cent produced more than 274 eggs during the contest year. These figures indicate a very satisfactory proportion of highly economical and profitable egg producers. The class between 200 and 224, inclusive, was the modal class and contained 949 individuals. The study dealt, therefore, with types of fowls which, other things being equal, might furnish at least a fair percentage of eligible breeders.

In the winter egg-yield distribution the modal class (10-egg interval) was that between 50 and 59, inclusive, with 652 cases, but the class next below the modal class and the two classes just above it were

close to it in frequencies, the four classes showing 558, 652, 639, and 577 cases, respectively. This span between 40 and 80 eggs as winter yield included 61.62 per cent of the total number of pullets in the flocks. This unusually high winter-production tendency was considered as indicating a generally high quality of birds in this study.

Further evidence of the high quality of these 3,937 birds was found in the third distribution curve, which indicated that as to "120+30" egg yield the modal class (again a 10-egg interval used) was that between 60 and 69, inclusive, with 604 cases, 66.32 per cent of the birds falling within a range between 50 and 100 eggs for this "120+30" period.

#### RELATION BETWEEN WINTER EGG YIELD AND ANNUAL TOTAL EGG PRODUCTION

The plotting of the means of each class interval in winter-yield distribution, as to annual egg production (fig. 1, B) at once intimated that a somewhat curvilinear relationship apparently existed between these two categories. The Blakeman test for linearity further con-

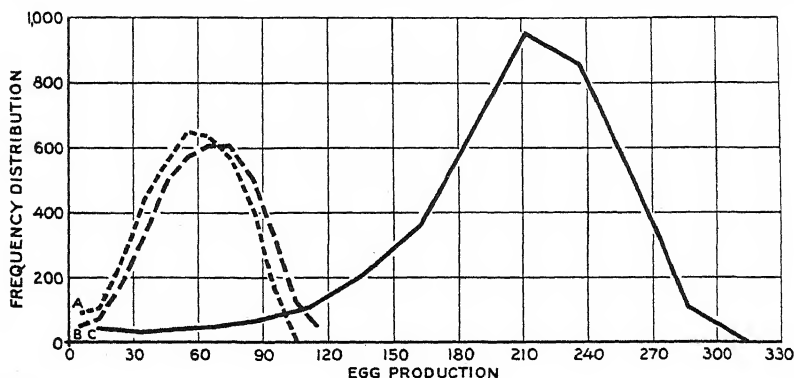


FIGURE 3.—Distribution of individuals (3,937 cases) as to egg production: A, winter egg production; B, first 120 plus last 30-day egg production; C, annual egg production

firmed this belief. Finally, the plotting of the regression lines (fig. 1, A and C, respectively) and a comparison of their locations with respect to the line connecting the means of the columns seemed to warrant the conclusion that in these 3,937 Single-Comb White Leghorn pullets the correlation between winter egg yield, as represented by the first 120-day egg yield, and the total egg yield for the first 51 weeks, or contest year, was distinctly curvilinear.

The correlation ratio, the  $\eta_{yx}$  value of  $+0.6642 \pm 0.0033$ , has been deemed a more accurate expression of the degree of relationship between these two categories than is the  $r_{xy}$ , or coefficient of correlation, of  $+0.6352 \pm 0.0064$ .

No very material advantage is to be gained through the use of the logarithmic-regression equation,  $\log Y = 1.24851 + 0.6143 \log X$ , over that of the straight-line equation,  $\bar{Y} = 1.553 X + 115.032$ , in the group having a winter egg yield approximately between 40 and 79 eggs, inclusive, since both equations would predict within a reasonable degree of the same thing. In those cases in which the winter yield is below 40 and above 80 eggs, the logarithmic prediction formula is more reliable than the straight-line equation.

It is not suggested that the formula presented necessarily gives the closest approach to the curvilinear data of this problem. Future studies of other possible formulas may determine a regression equation which will adequately fit the data presented. At the moment, with particular emphasis upon practical usage, the use of the 2-regression equation as indicated in the text and in Figure 2 is offered as a workable proposition at least pending the finding of some single-regression equation which in itself would more nearly fit the data.

The author feels so strongly that the 3,937 cases used in this investigation represent a fair cross section that he would be inclined to use the graphic method of predicting annual egg production from known winter yield rather than either of the mathematical formulas here presented. He suggests that the curve shown in Figure 1, B might be thus used advantageously. The practical poultry breeder, on January 28 would stop trap nesting and recording and add up the individual records for the winter months. Then, having for each individual bird a known winter-season egg-yield record he would find that known record along the x, or base line. The intersection of the line from that point with curve B, in Figure 1, indicates the predicted or expected annual egg production. For example, if the known winter yield is 60 eggs, one reading direct from the graph would predict an annual production of 210 eggs, if the winter yield is 40 eggs, the annual production would be about 180 eggs, and if the winter yield is 75 eggs, the annual production would be about 230 eggs, etc.

If the winter egg yield may be taken as a reasonably fair indication of the inherited capacity of fowls to produce eggs, the economic significance of this winter egg-yield record is at once apparent. It permits the poultry breeder to cease the expensive and laborious task of trap nesting and recording after January 28. It furnishes him with sufficiently accurate and complete information concerning each individual to allow him to group or classify his fowls with a high degree of skill. Upon this premise it is suggested that the average flock thus trap nested during only the 120-day period after October 1 might be divided into three groups, for breeding purposes, as follows:

Group 1 would include pullets producing from 50 to 69 eggs, inclusive, during their first 120 days, a class with a range of predicted annual egg-production values from approximately 196 through 240 eggs (using logarithmic equation). All birds that do not come up to this minimum of 50 eggs during the winter period should be discarded.

Group 2 would include those pullets producing from 70 to 89 eggs, inclusive, a class with predicted annual egg-production totals between approximately 240 and 280 eggs.

Group 3, the smallest group, in numbers, would include those pullets producing more than 89 eggs during the winter period, or having a predicted annual record of 280 or more eggs. This group would represent the highest degree of laying efficiency found in the trap-nested stock.

Of course, such selection and grouping as is here suggested would be made only if other things were equal, such as health, type, and size, and, perhaps, standard quality.

If the trap nesting must stop with the winter season, this plan is workable.

## RELATION BETWEEN "120+30" EGG YIELD AND ANNUAL PRODUCTION

Hervey (7) found a significant correlation between the summer-fall period egg yield and the total egg production for the year, expressed by the coefficient  $+0.7860 \pm 0.0135$ . Other investigators have indicated that prolonged production throughout the pullet year period was an indication of high egg-yield capacity. As explained earlier in this paper, the arbitrary 30-day period between August 25 and September 23 has been chosen as a measure of prolonged egg yield. It was suggested that this measurement added to that of the winter season might furnish a still better criterion of selection than did winter yield alone, or summer-fall record alone.

From a practical point of view, this seems sound. The poultry breeder can economically and easily trap nest a fairly large number of pullets during the first four months, or through January 28. He must then stop that work because of the heavy demands of the chick hatching and rearing season. He must cut down on expenses as well.

As summer wanes, however, the young chickens need little care. The poultryman's time is less filled and he finds that he can again take up some trap nesting. He will start trap nesting the new pullets as October approaches. He can easily start to operate the trap nests on the laying flocks during late August and economically and efficiently trap nest some of the pullets which he trap nested during the preceding winter season.

The second part of this investigation consisted in an attempt to find out whether or not it would be worth while to trap nest during this late 30-day period. By similar tests to those applied to the winter-season data, the relationship between the "120+30" day egg yield and annual egg production was found to be distinctly curvilinear, as shown by Figure 2.

A study of the three curves in Figure 2 reveals a considerably more interesting and probably a much more serviceable measurement of egg-production capacity than was found when only the winter egg yield was used. In the first place, the degree of association between the "120+30" day egg-yield record and that of the full year is greater than that in the other case. In this instance the value of  $r$  was  $+0.6711 \pm 0.0088$ , and that of  $\eta_{yz}$  was  $+0.7150 \pm 0.0078$ . This gives an increased value to the predictions based upon that relationship. The curvilinear nature of the relationship is sharply defined, and the line connecting the means of columns tends to coincide with the two regression lines.

After a careful study of Figure 2 and its formulas, used on innumerable cases for prediction purposes, the following suggestions are offered: (1) When the egg yield during the "120+30" period is below approximately 42 eggs, the relationship which that yield bears to the annual total egg production is very distinctly curvilinear in character. The predicted annual production attained by use of the logarithmic formula,  $\log Y = 1.2324 + 0.64208 \log X$ , and that obtained by direct reading through use of curve B, in Figure 2, are very closely similar. The differences are, at least from the standpoint of the practical poultry breeder, negligible. (2) A division line can be established, therefore (*aa* in fig. 2), below which point the logarithmic equation is to be used, if for any reason it is desired to predict annual production on birds of that degree of egg-laying capacity. (3)

Above the line *aa* the straight line equation,  $Y = 0.8138X + 153.2667$ , should be used for prediction purposes, or a direct reading from curve B, either of which would render approximately the same results.

Although for the practical poultryman interested primarily in finding a method of using a short-period trap-nest record as a criterion of selection, the linear equation just referred to would be sufficient, it is of interest to poultry science to have the situation as it stands below the *aa* line defined.

The foregoing discussion has been limited to the possible statistical and economical usefulness of (1) the winter egg-yield record alone, and (2) the combination of the winter record with that of the last 30 days of the laying year, i. e., the "120+30" period record. It is possible that, if, instead of combining the winter and the last 30-day records into one measurement, each is used separately, still closer prediction of annual egg production might be made. Therefore, the study made as problem 2 was undertaken. A partial correlation was worked out, and a partial regression equation evolved. The separate use of the two short-period trap-nest records offers a more accurate and exact means of prediction than does either of the other two formulas and is no more difficult to use. Scientifically, it is, perhaps, an interesting and worth-while example of the usefulness of the partial correlation and regression principle to poultry-science problems.

#### SUMMARY

In the case of 3,937 Single-Comb White Leghorn pullet layers studied as to first-year egg-production capacities, a highly significant positive correlation of curvilinear type was found to exist between the egg yield during the first 120 days after October 1 (approximately date of first egg) and total egg production for the contest year (51 weeks after October 1).

A still more significant positive curvilinear correlation existed between the "120+30" day egg yield and annual total egg production.

If both winter egg yield and yield during the 30-day summer period are available, the practical poultry breeder has an efficient means of predicting annual total egg-producing capacity in the partial regression, or prediction equation here given.

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# INHERITANCE IN ALBIT WHEAT OF RESISTANCE TO BUNT, *TILLETIA TRITICI*<sup>1</sup>

By E. N. BRESSMAN, *Associate Agronomist*, and L. E. HARRIS, *Assistant, Oregon Agricultural Experiment Station*

## INTRODUCTION

Albit wheat (Washington No. 2517, C. I. No. 8275, and registration No. 258) is the result of a cross between Hybrid 128 (female) and White Odessa (male), made in 1920 by E. F. Gaines. It is a club wheat with awnless spikes, glabrous white glumes, and soft white kernels.<sup>2</sup> In morphological characters it is like Hybrid 128<sup>3</sup> (fig. 1), but in its resistance to bunt (*Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn) it resembles White Odessa.

In 1931 the senior author<sup>4</sup> reported that Albit showed the same reaction to bunt as White Odessa, its bunt-resistant parent. "It appears," he stated, "that Gaines has completely transferred the resistance of White Odessa to a segregate having morphological characteristics similar to those of the susceptible parent, Hybrid 128."

Martin, White Odessa, Banner Berkeley, Albit, and Regal wheats have been found by the senior author<sup>5</sup> to be similar in their resistance to *Tilletia tritici* and *T. levis*. Briggs<sup>6</sup> has shown that Martin, White Odessa, and Banner Berkeley have one main dominant factor for resistance to *T. tritici*.

## EXPERIMENTAL DATA

Data previously reported by the senior author<sup>7</sup> showing the reaction of White Odessa and Albit wheats to 43 different collections of bunt are presented graphically in Figure 2. This figure shows that the two varieties are similar in their reaction to all 10 of the forms of bunt (included in collections) used in earlier tests. Both were resistant to 29 collections and susceptible to 14. The one discrepancy, apparent susceptibility of White Odessa to collection 7c and resistance of Albit, was cleared up in 1929 when it was found that both varieties are resistant to this collection. Other trials in 1930 and 1931 also indicate that these two varieties are identical in their ability to resist bunt.

In 1928 several crosses were made between Hybrid 128 and Albit. The F<sub>1</sub> plants were grown in the greenhouse in 1928-29. The F<sub>2</sub> plants were grown in 1929-30 from copper-carbonate treated seed so that the F<sub>3</sub> generation should contain both susceptible and resistant

<sup>1</sup> Received for publication Apr. 13, 1932; issued March, 1933. Contribution from the Department of Farm Crops, Oregon Agricultural Experiment Station. Paper No. 183 of the Journal Series.

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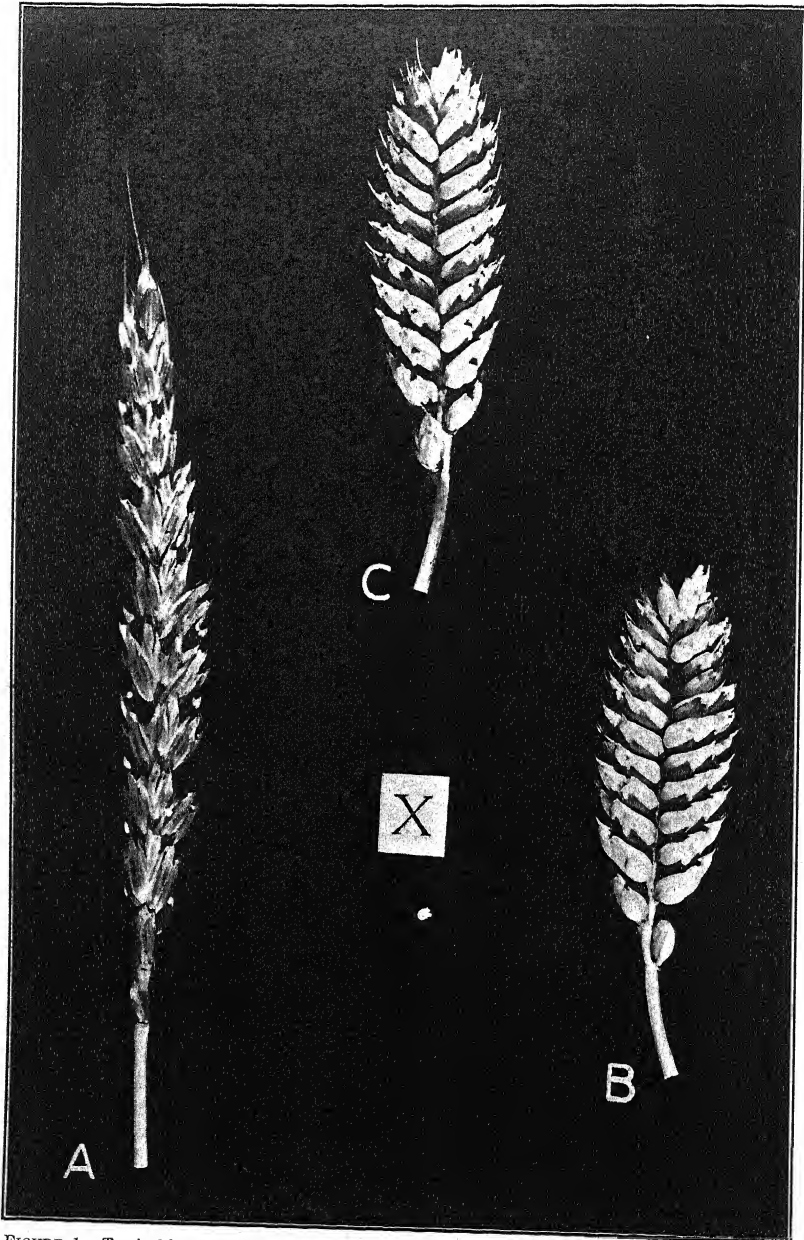


FIGURE 1.—Typical heads of White Odessa (A) and Hybrid 128 (B). A cross between these two, made by E. F. Gaines in 1920, yielded a segregate, Albit (C), which carries the morphological characters of the female parent (Hybrid 128) and the bunt resistance of the male parent (White Odessa)

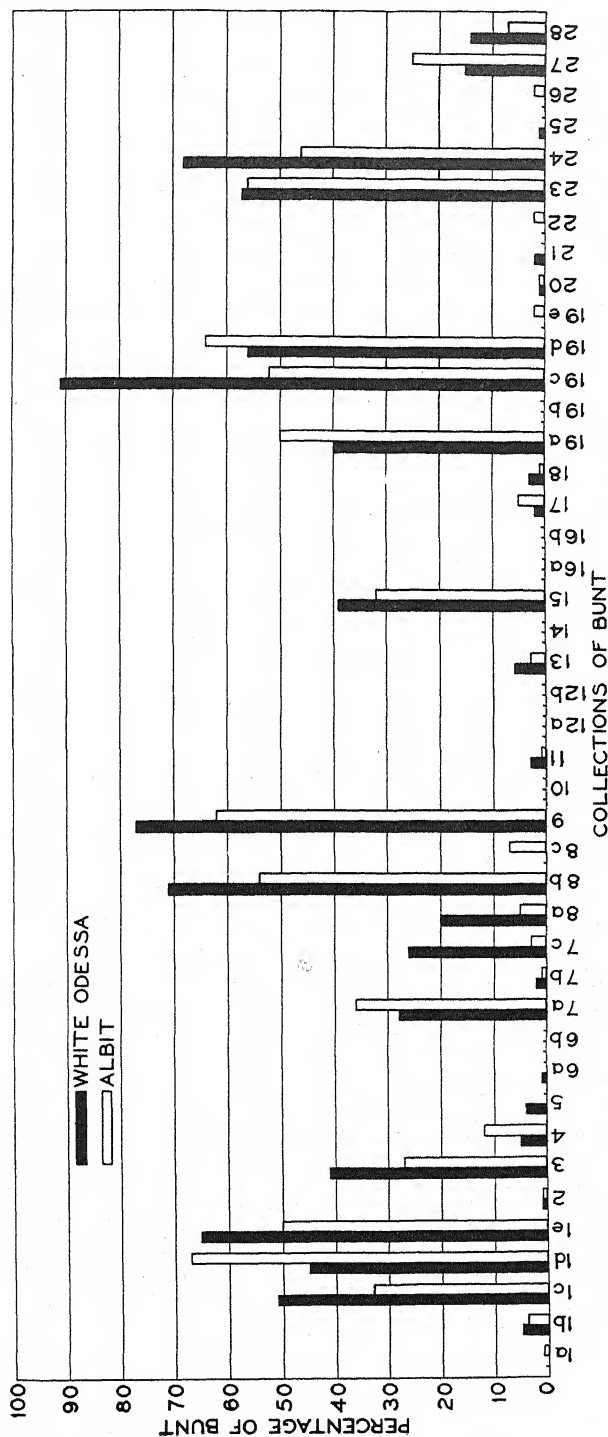


FIG. 2.—Reaction of White Odessa and Albit wheats to various collections of bunt. The percentages of bunt are the averages of two trials conducted in 1928 at Corvallis and Moro, Oreg. Note the similarity of reaction of these two varieties, parent and segregate

segregates. In the fall of 1930 the seed for the  $F_3$  generation was heavily coated with inoculum from bunt collection No. 12a. This collection was originally obtained from Tucson, Ariz. It is a form of *Tilletia tritici*, classified as physiologic form VIII. This collection was used as inoculum because the Hybrid 128 parent is susceptible and the Albit parent is resistant.

No bunt appeared at Corvallis on Albit when it was inoculated with collection 12a (Table 1); but in 1929 at Moro 2 bunted heads out of a total of 505 were found in this variety. No significance is attached to this fact, however, for a few bunted heads are commonly found in a resistant variety. Hybrid 128 in all tests was susceptible to collection 12a. At Moro in 1929 this collection produced 63 per cent infected heads in Hybrid 128.

White Odessa was inoculated with collection 12a in 1929 only. In these trials 200 heads of White Odessa were counted at Corvallis and 300 at Moro, but no bunt was found.

TABLE 1.—Percentage of bunt in the parent varieties when inoculated with bunt collection 12a, Corvallis, Oreg.

Variety	Percentage of bunt in—				
	1928	1929	1930	1931	Average
Albit	0	0	0	0	0
Hybrid 128	43	40	91	82	64

In the Hybrid 128  $\times$  Albit crosses, the  $F_1$ ,  $F_2$ , and  $F_3$  progenies were all identical in morphological characters with the parent varieties. In other words, there was no segregation for these characters. In fact, the only method of determining that hybridization had been effected was through the segregation for bunt resistance. This segregation is shown in Table 2. Each row, fall sown in the field at Corvallis, was 16 feet long and contained about 60 plants. The  $F_3$  generation was represented by four families and a total of nearly 5,000 plants. One family did not segregate for bunt resistance, indicating that it was not a hybrid. The results of its smutting, therefore, are not included.

TABLE 2.—Distribution of the  $F_3$  rows of the cross, Hybrid 128  $\times$  Albit, into 10 per cent classes for bunt infection, *Tritici* species, collection 12a, physiologic form VIII, Corvallis, Oreg., 1931

Hybrid family No.	Number of rows having indicated percentage of plants infected										Total rows
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
1	5	2	5	1	2		1	4	4		24
2	3		1			1	2	1			8
3	4	3	1	4					2	2	16
4	4	4	7	5	1			2	3	2	28
Total	16	9	14	10	3	1	3	7	9	4	76

$P=0.72$ .

In the crosses between Hybrid 128 and Albit the distribution of  $F_3$  rows into 10 per cent classes for bunt infection agrees closely with a 1:2:1 ratio when the 0-10 class is considered as resistant and the classes above 70 as susceptible. In the resistant class there are 16 rows, in the susceptible class 20, and in the segregating class 40. On the basis of a 1-factor difference, there should have been 19, 38, and 19 rows, respectively, in the three classes. Since  $P=0.72$ , a very satisfactory agreement with the 1:2:1 ratio is indicated. The value of  $P$  was obtained from Fisher.<sup>8</sup>

One family, No. 3, shows a perfect segregation of 1:2:1 with 4 resistant rows, 8 segregating, and 4 susceptible in a total of 16 rows. In fact, all four families show a satisfactory agreement with a 1:2:1 ratio. The  $F_3$  rows are plant rows.

Briggs<sup>9</sup> states that there may be more than one factor for resistance in White Odessa wheat. The results herein reported indicate that White Odessa has only one factor for resistance and that it applies only to certain physiologic forms of bunt, namely, Nos. II, III, V, VI, and VIII of the senior writer's classification.<sup>10</sup> This factor ( $MM$ ) has been transferred from White Odessa to Albit, so that both varieties contain the identical factor. These varieties, however, contain no factor for resistance to forms I, IV, VII, IX, and X. Hybrid 128 is entirely susceptible and carries no factor for resistance to any of the physiologic forms.

#### SUMMARY AND CONCLUSIONS

Albit wheat contains one main dominant factor for resistance to certain physiologic forms of bunt. This factor,  $MM$ , is the same as that carried by its male parent, White Odessa.

Apparently the factor  $MM$  is fairly well distributed, for it has been found by Briggs (who designated it) or by the writers in Martin, White Odessa, Hussar, Banner Berkeley, Albit, and possibly Regal wheats. These wheats differ in species, size and shape of head, color of kernel, color of chaff, bearded condition, color of straw, and other characters.

Albit wheat contains morphological characters similar to its female parent, Hybrid 128. Crosses between Albit and Hybrid 128 do not segregate for morphological characters, but they do segregate for bunt resistance and possibly for other factors such as yielding ability. Apparently Hybrid 128 carries no factor for bunt resistance.

<sup>8</sup> FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p., illus. Edinburgh and London. 1925.

<sup>9</sup> BRIGGS, F. N. Op. cit. (Footnote 6, first reference.)

<sup>10</sup> BRESSMAN, E. N. Op. cit.



# THE VITAMIN C CONTENT OF THE WINESAP APPLE AS INFLUENCED BY FERTILIZERS<sup>1</sup>

By MYRA T. POTTER, *Instructor in Foods and Nutrition, State College of Washington*, and E. L. OVERHOLSER, *Horticulturist, Washington Agricultural Experiment Station*<sup>2</sup>

## INTRODUCTION

The value of many fruits as a source of vitamin C is widely accepted. Much information is available concerning the occurrence and stability of vitamin C in food materials. Information is incomplete, however, concerning the possible influence of various factors in the field, such as cultural operations employed in growing the product. The application of mineral fertilizers may measurably affect tree vigor, the relation of leaf area to fruit borne, the size, color, and time of maturity of individual specimens, and even their chemical composition, especially the mineral content.

Bracewell, Hoyle, and Zilva (2)<sup>3</sup> found that varieties were significantly different in vitamin C potency, but they reported no indication that the age of trees, soils, or season had any bearing on the antiscorbutic potency of the apple. A publication from this same laboratory in 1931 (4), upon vitamin C and the nitrogen content of apples, reported no conclusive evidence of a relationship between nitrogen content and vitamin C value.

It was believed that a study of the possible influence of the application of a complete fertilizer (N, P, and K) upon the vitamin C content of the apple, as contrasted with apples from trees receiving no fertilizer, would be of interest. As a basis for this study, the Winesap variety, found by Potter (9) to be a good source of vitamin C, was selected since the minimum protective level had been established.

## MATERIALS AND METHODS

In an attempt to obtain more definite information on the possible influence of applying complete fertilizers upon the vitamin C content of Winesap apples, fruit was obtained from fertilizer experimental plots of the Divisions of Horticulture and Agronomy, Washington Agricultural Experiment Station, in the Wenatchee district. The trees were 20 years old and were growing in a Cashmere gravelly coarse sandy loam (Kocher (6)). The orchard was in cover crop, consisting of a poor stand of alfalfa with considerable weed growth, and was irrigated by the rill or furrow system.

The fertilized trees during the years 1928 to 1931, inclusive, received annual applications per tree of a complete fertilizer as follows: 5.7 pounds of an equal mixture of ammonium sulphate and

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<sup>2</sup> Appreciation is expressed to Leila W. Hunt, professor of foods and nutrition, College of Home Economics, for helpful suggestions and aid in the nutritional phases of this study; to Prof. F. L. Overley of the Division of Horticulture, who was responsible for the application of the fertilizers and who harvested the apples utilized.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 373.

sodium nitrate, 11.7 pounds superphosphate, and 3.9 pounds of a mixture of equal parts of muriate of potash and sulphate of potash. These fertilizers were broadcast in December either on the ground surface or on the snow. The check trees received no fertilizer applications.

Comparable samples of Extra Fancy grade of the commercial size of Winesap apples, averaging 125 specimens per box, were harvested from both plots at the commercial stage of maturity, and immediately forwarded to Pullman, where they were retained at temperatures of 40° to 45° F. The vitamin C studies which were initiated shortly after the fruit was received, were conducted with guinea pigs, using essentially the technic of Sherman, LaMer, and Campbell (10).

The guinea pigs were observed in the laboratory for at least one week before beginning the experiment, in order to select normally growing animals. During this period the basal vitamin-C-free ration was fed, supplemented with vitamin-C-rich foods, to adjust the animals to the basal ration. This basal ration was that of Sherman, et al. (10) with several modifications:

	Per cent
Oats, sound whole grain, ground.....	58
Baked skim-milk powder (heated for 4 hours at 110° C.).....	30
Butterfat, filtered and freshly prepared.....	10
Sodium chloride.....	1
Cod-liver oil.....	1
	100

Forty-two guinea pigs, averaging approximately 325 g<sup>4</sup> each were used for this experiment, the animals being divided as follows:

	Number of animals
Basal ration, plus orange juice (positive control).....	6
Basal ration only (negative control).....	6
Basal ration, plus apple:	
5 g fertilized Winesap apple.....	15
5 g nonfertilized Winesap apple.....	15
(Apple feedings made daily, 6 days per week.)	

The animals were kept individually in galvanized screen cages with raised false bottoms. Fresh water and the basal ration were supplied daily.

The guinea pigs were observed daily for lameness, soreness, and enlargement of wrists, and were weighed on alternate days. The experimental periods used during 1930-31 were of 70 to 90 days' duration. Since Morgan et al. (8) presented data which show the adequacy of a shorter experimental period, the interval employed during 1931-32 was 56 days.

The positive-control animals were given orange juice as their source of vitamin C, this being fed at a level well above the generally accepted protection requirement.

The apple feedings were prepared each day following a definite technic. Vitamin C is easily destroyed by oxidation, as has been demonstrated by Kohman (7) and Kenney,<sup>5</sup> and other workers. Hence, a method was developed by which a uniform sample of apple pulp could be prepared quickly with minimum oxidation resulting.

<sup>4</sup> g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

KENNEY, C. L. A STUDY OF THE THERMOSTABILITY OF VITAMIN C. 1926. (Diss., Columbia Univ.)



The apples were wiped, cored, quartered, and the skin surfaces scarified about one-eighth inch apart. The apple tissues were then ground, weighed, and fed to the animals immediately. Such a method produced a composite sample, with the skin and subepidermal tissues uniformly distributed. According to Bracewell, Kidd, West, and Zilva (3), these tissues contain the greatest concentration of vitamin C. Furthermore, the feedings were consumed by the animals before there was appreciable evidence of brown discoloration from oxidation.

At the close of the experimental period, autopsy studies were made on all animals, the Sherman scurvy score (10) being used to evaluate and record the findings. With this method of scoring, 0 indicated an absence of apparent evidence of scurvy while a score of 24 signified a condition of severe scurvy in all of the enumerated points of examination. The range from 1 to 24 was divided into four classes, with an interval width of 6. These classes designated the following states of scurvy: 1-6, mild; 7-12, moderate; 13-18, severe; and 19-24, very severe.

In expressing autopsy findings a negative sign indicated absence of scurvy, while 1 to 3 plus signs showed an increasing degree of severity of scurvy. It was thought that possible differences in vitamin C value between fruit of apple trees receiving complete fertilizers and apples from trees not fertilized would be more apparent when the animals were fed quantities slightly below the protective dose. Potter (9) found that Winesap apples, whether from fertilized or non-fertilized trees, when fed in 10 g amounts daily protected guinea pigs from scurvy and allowed them to gain in weight. The animals receiving the 5 g feedings, however, exhibited scurvy in all but a few cases. This smaller dosage of apple, therefore, seemed to offer the degree of protection where small differences in vitamin C content could be discerned.

#### EXPERIMENTAL RESULTS

The experimental data are shown in Table 1.

TABLE 1.—Results of feeding Winesap apples from fertilized and from unfertilized trees to guinea pigs as the sole source of vitamin C

POSITIVE CONTROLS FED BASAL DIET AND ORANGE JUICE

Animal No.	Body weight			Duration of experiment	Autopsy findings <sup>a</sup>								Scurvy score	Final scorbutic condition	
	Initial	Maxi- mum	Final		Average daily loss or gain	Bony system				Hemorrhages					
						Teeth	Joints	Ribs	Bones	Ribs	Intes- tines	Joints			Muscles
	Grams 300	421	421	Grams +1.3	—	—	—	—	—	—	—	—	—	0	No scurvy.
	297	448	448	Days 90	—	—	—	—	—	—	—	—	—	0	Do.
	285	452	452	+1.7	—	—	—	—	—	—	—	—	—	0	Do.
	295	546	546	+2.4	—	—	—	—	—	—	—	—	—	0	Do.
	304	469	469	+3.6	—	—	—	—	—	—	—	—	—	0	Do.
	364	469	469	+1.9	—	—	—	—	—	—	—	—	—	0	Do.
	331	501	493	+2.9	—	—	—	—	—	—	—	—	—	0	Do.
Average	312	473	471	+2.3	0	0	0	0	0	0	0	0	0	0	

NEGATIVE CONTROLS FED BASAL DIET ONLY

5.....	310	310	179	25	++	++	++	++	++	++	++	++	21	Very severe.
11.....	297	297	215	21	++	++	++	++	++	++	++	++	14	Severe.
17.....	301	301	155	26	++	++	++	++	++	++	++	++	20	Very severe.
18.....	290	290	152	22	++	++	++	++	++	++	++	++	20	Do.
43.....	281	281	142	35	++	++	++	++	++	++	++	++	20	Do.
60.....	323	323	176	26	++	++	++	++	++	++	++	++	16	Severe.
Average.....	300	300	170	26	2.8	2.8	2.5	2.5	1.8	2.2	2.0	1.8	19	



The positive-control animals lived throughout their experimental periods, and were protected from scurvy. The average weight gains for this group were 2.3 g daily. The negative-control group, however, lost 5.1 g daily, and had an average survival period of only 26 days. These negative animals showed a very severe scorbutic condition at autopsy as the high average scurvy score of 19 indicated. Their life period on the scorbutic basal diet alone corresponds very closely with the data of Sherman, LaMer, and Campbell (10).

In Table 1, data are also presented for the guinea pigs that were fed 5 g amounts of the Winesap apples from trees receiving applications of the complete fertilizer. Three animals died from scurvy before the end of their experimental period. The average scurvy score for the group was 6, which indicated a mild scorbutic condition. The average daily weight loss was 1.1 g.

The records for the guinea pigs that were fed 5 g of nonfertilized Winesap apples as their source of vitamin C are likewise shown in Table 1. In this group four animals died before the experimental period terminated, and had marked scorbutic lesions. Most of the animals lost weight (an average loss of 1.6 g daily) and those surviving the experimental period with only three exceptions showed marked evidences of scurvy at post-mortem examinations. The average scurvy score for this group was 10.6 which would give the group an average rating of moderate scurvy.

#### DISCUSSION OF RESULTS

Bracewell, Wallace, and Zilva (4, *p.* 146) summarized their findings concerning the relation of potency of vitamin C to nitrogen content of apples as follows:

King Edwards containing about 0.0307% of nitrogen were about 1.5 times as potent antiscorbutically as apples of this variety containing about 0.0387% of nitrogen. No significant disparity in the vitamin C content was found between Bramley's Seedlings containing high and low quantities of nitrogen.

In view of these results it would have been of interest to know the nitrogen content of the fertilized and nonfertilized Winesap apples employed in the experiment herewith reported. Such analyses, however, are not available. According to Gourley and Hopkins (5), and Aldrich (1), the application of nitrogen-carrying fertilizers tends to increase the nitrogen content of the apples of trees so fertilized. It is of course possible that some one of the other elements in the complete fertilizer applied, rather than nitrogen, may have been responsible for differences observed in vitamin C potency between the fertilized and unfertilized Winesap apples, and as herewith reported. It is also possible that the effect may have been an indirect one rather than any increase in the actual mineral content of the apple.

#### CONCLUSIONS

The fact that 60 per cent of the animals receiving apples from fertilized trees were protected or developed only mild scurvy, while with the animals fed apples from nonfertilized trees not one was protected and 80 per cent developed moderate to very severe scurvy, is significant.

From the data submitted, Winesap apples from trees receiving applications of a complete fertilizer appeared to be a better source of vitamin C than apples from trees not so fertilized, when fed at the

5 g level. This should not be interpreted as indicating that Winesap apples are an unsatisfactory source of vitamin C unless from trees receiving applications of a complete fertilizer. Potter (9) has found that when fed at the 10 g level, differences were not apparent between fruit from fertilized and from nonfertilized trees.

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# YIELD AND CHEMICAL COMPOSITION OF CERTAIN PASTURE CROPS, FERTILIZED AND UNFERTILIZED <sup>1</sup>

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## INTRODUCTION

The high yields of pasture herbage obtainable in the valleys of western Washington make it desirable to secure data relative to the value of adapted grasses, and the changes that occur as a result of fertilizer applications or improved methods of management.

Unpublished data secured by McCollam <sup>2</sup> in 1928 and by the author in 1929 showed that yields ranging from 6,000 to 10,000 pounds of dry matter per acre could be obtained from pasture crops grown on moist bottom-land soil, when the crops were cut at intervals of approximately a month. It was found, however, that with a growing period of a month, the grasses reached a comparatively advanced stage of maturity. In the work here reported, a 14-day growth period was allowed between cuttings in place of the monthly period previously employed, since it was desired to obtain information relative to the composition and yield of pasture crops in the more immature stages.

## WORK OF PREVIOUS INVESTIGATORS

McCollam, <sup>2</sup> at the Western Washington Experiment Station, in 1928 recorded yields on a number of pasture crops growing on a muck soil well supplied with moisture. A bottom-land mixture consisting of adapted grasses and clovers recommended for western Washington (8), <sup>3</sup> cut on the specified dates, produced the following yields of dry matter in pounds per acre: April 27, 2,375.7 pounds; June 5, 2,544.6 pounds; July 9, 2,252.9 pounds; August 4, 1,659.4 pounds; and August 25, 861 pounds. The total yield for the season was 9,693.6 pounds of dry matter per acre. During the same period Italian ryegrass produced 8,697.8 pounds of dry matter per acre, and white clover 6,685.8 pounds of dry matter per acre.

Woodman, Norman, and Bee (14) analyzed samples of a mixed grass cut at intervals of three weeks from April to October, 1929. They found that the average protein content of the grass cut in this manner was 21.14 per cent, as compared with 23.48 per cent for fortnightly cuttings made in 1927 and 24.74 per cent for weekly cuttings made in 1925.

Ellenberger, Newlander, and Jones (2) secured from frequently clipped pasture plots (1925 and 1926) an average yield of 1,748 pounds of dry matter per acre. The average composition of the clippings was: Crude protein, 20.30 per cent; crude fiber, 18.69 per cent; crude ash, 11.68 per cent; nitrogen-free extract, 46.03 per cent;

<sup>1</sup> Received for publication May 4, 1932, issued March, 1933.

<sup>2</sup> MCCOLLAM, M. E., unpublished data.

<sup>3</sup> Reference is made by number (*italic*) to Literature Cited, p. 386.

ether extract, 3.3 per cent; calcium, 0.816 per cent; and phosphorus, 0.32 per cent. They concluded (2, p. 65) "the nutrients in closely grazed pasture more nearly resemble a concentrate than a roughage."

Hopper and Nesbitt (7) were able to maintain a high percentage of protein throughout the season in pure stands of brome grass, slender wheatgrass, and crested wheatgrass, by cutting twice monthly.

Shutt, Hamilton, and Selwyn (10) in 1927 obtained an average protein content of meadow foxtail (*Alopecurus* sp.) of 21.20 per cent (dry-matter basis) for plots cut weekly, 18.60 per cent for plots cut fortnightly, and 17.17 per cent for plots cut every three weeks. The seasonal yields of dry matter for cuttings at intervals of 1, 2, and 3 weeks were 2,918, 3,344, and 4,304 pounds, respectively.

Archibald and Nelson (1) found that an application of complete fertilizer followed by later applications of nitrogenous fertilizer on a rotationally grazed pasture decreased considerably the percentage of dry matter in the grass, and decreased slightly the percentage of crude fiber and calcium in the dry matter. The percentage of nitrogen, phosphorus, and ether extract was increased, the nitrogen markedly so.

Greenhill (5) found that the average protein content of rotationally grazed pasture was increased from 22.6 per cent to 25.3 per cent by intensive fertilization with nitrogenous fertilizers.

Enlow and Coleman (3, p. 852) concluded: "The protein content of pasture in a grazed condition can be increased and maintained at a somewhat higher level than ordinary by frequent light applications of a nitrogen fertilizer."

#### METHOD

The plots used in this experiment are located on a low-lying tract of valley land belonging to the Puget series (sandy loam). During the winter months the water table lies approximately 18 inches below the surface. Following periods of exceptionally heavy rains, however, the water table frequently comes to within a few inches of the surface for short periods. In the dry months of late summer, the water table falls to a depth in excess of 3 feet. The soil used in this experiment is typical of considerable areas of alluvial soil lying in the valleys of the Puget Sound Basin. While the soil is productive, crops are often limited in their growth by lack of moisture during the summer and fall months.

The area had previously been in pasture for six years, and had received during that period, one or two light applications of barnyard manure. The latest manure application had been made during the winter of 1928-29.

The land was plowed during the winter previous to seeding the plots. The area was disked and harrowed a number of times before the final preparation of the seed bed. After the ground had been thoroughly prepared, twelve  $\frac{1}{10}$ -acre plots were staked out. Six of these plots received the following fertilizers:<sup>4</sup> N, 45 pounds;  $P_2O_5$ , 87.5 pounds; and  $K_2O$ , 100 pounds per acre.

The carriers employed were sodium nitrate, superphosphate, (17.5 per cent  $P_2O_5$ ), and potassium chloride. Hydrated lime ( $Ca(OH)_2$ ) at the rate of 2,000 pounds per acre was also applied to

<sup>4</sup> The application of fertilizers and hydrated lime was made at rates suggested by Henry Holtz, soils section, department of agronomy, State College of Washington.



the plots receiving the fertilizer. The fertilizers applied just before the plots were seeded were worked into the topsoil with a hand rake.

Two pasture crops (Italian ryegrass and white clover) seeded alone, and one mixture were used in this experiment. The mixture was the bottom-land mixture recommended for western Washington by McCollam (8). This mixture was made up of grasses and clovers in the following proportions by weight: Italian ryegrass, 3; English ryegrass, 3; meadow fescue, 4; tall meadow oatgrass, 2; seaside bent grass, 3; common white clover, 4; red clover, 2; and alsike clover, 1. Duplicate  $\frac{1}{100}$ -acre plots of each crop were seeded on both fertilized and unfertilized areas, between March 15 and 20, 1930.

Rapid growth was made on each plot, and on May 12, a preliminary cutting of the Italian ryegrass and bottom-land mixture was made. The preliminary cutting of the white clover was made on May 19. A lawn mower was used throughout the season for cutting the plots. The cutting bar of the lawn mower was set to cut at a height of  $1\frac{1}{4}$  inches.

The preliminary cutting was discarded. Succeeding cuttings of the plots were made at regular intervals of 14 days. The green material from the duplicate  $\frac{1}{100}$ -acre plots of each crop was combined at the time of cutting and thereafter was considered as the yield from one-hundredth of an acre. The regular biweekly cuttings were continued through December, 1930. Immediately after being cut, the fresh grass was weighed in the laboratory, to obtain the green weight, and thoroughly mixed. Duplicate 100-g<sup>5</sup> samples were then taken from each lot. These samples were put in muslin bags and dried<sup>6</sup> until no further change in weight occurred.

The average percentage of dry matter of the duplicate 100-g samples was taken as the percentage of dry matter for the entire sample. The remainder of the material from each cutting was also dried and preserved for analysis. One sample for analysis was obtained each month from each plot. This sample was prepared by thoroughly mixing all the dry material from cuts made on one plot during one month. A portion of this material was then analyzed.<sup>7</sup>

#### PRESENTATION OF DATA AND DISCUSSION OF RESULTS

Table 1 shows that the year 1930 was unusually dry, the total rainfall being 9.33 inches less than the average for the previous 10 years.<sup>8</sup> A fair amount of rain fell until June 7, but from that date until late September, the rainfall was far below normal. From June 8 to September 26, a period of over three and one-half months, the total rainfall was only 1.05 inches. The rainfall was 0.84 inch and 0.72 inch above normal in May and June, respectively, but that for July, August, and September was 0.50, 1.06, and 0.97 inches, respectively, below normal. On September 27 a heavy rain fell, and thereafter the rainfall was more abundant. Table 1 indicates that the precipitation for July, August, and September was very unfavorable to the growth of pasture grasses.

<sup>5</sup> g is the abbreviation for gram or grams recently adopted by the Style Manual for United States Government printing.

<sup>6</sup> The temperature of drying was approximately 85° C. It was impossible with the oven used, to keep the temperature entirely uniform; the range was from 80° to 95°.

<sup>7</sup> The samples were analyzed by Clarence Kaseline in the laboratories of the division of chemistry of the agricultural experiment station.

<sup>8</sup> From the records obtained at the U. S. Weather Bureau station located on the grounds of the Western Washington Station.

Table 1 also shows that the temperature during the 1930 growing season, as compared with the average for the previous 10-year period, was favorable to growth. The temperature during July, August, and September averaged higher than that during May and June. In spite of this fact, production decreased during July, August, and September because of unfavorable moisture conditions.

TABLE 1.—Rainfall and mean monthly temperatures for 1930, as compared with averages for the previous 10-year period, 1920-1929

RAINFALL (INCHES)													
	Janu- ary	Febru- ary	March	April	May	June	July	Aug- ust	Sep- tem- ber	Octo- ber	Nov- ember	Dec- ember	An- nual
Average for 1920-1929.....	5.67	3.38	3.50	2.54	2.18	1.06	0.50	1.20	2.00	3.68	4.38	5.87	35.96
1930.....	2.15	5.46	2.97	2.22	3.02	1.78	0	.14	1.03	2.43	3.26	2.17	26.63
Deviation in 1930.....	-3.52	2.08	-.53	-.32	.84	.72	-.50	-1.06	-.97	-1.25	-1.12	-3.70	-9.33

MEAN MONTHLY TEMPERATURES (°F.)													
	Janu- ary	Febru- ary	March	April	May	June	July	Aug- ust	Sep- tem- ber	Octo- ber	Nov- ember	Dec- ember	An- nual
Average for 1920-1929.....	38.6	40.8	44.8	48.6	54.5	60.1	63.6	66.4	61.8	56.3	44.2	39.5	45.3
1930.....	29.0	43.4	46.2	51.8	53.8	59.0	62.8	64.8	58.8	48.8	43.0	39.4	50.1
Deviation in 1930.....	-9.6	2.6	1.4	3.2	-.7	-1.1	-.8	8.4	7.0	2.5	-1.2	-.1	4.8

Table 2 shows that the highest yields of dry matter in all plots were obtained during June. Beginning late in June or early in July, there is a decrease in the production of all plots to the end of the year. There seems to be little difference between the fertilized and the unfertilized plots in this respect, although the production of the fertilized plots was usually at a higher level.

TABLE 2.—Yield of dry matter in pounds per acre, and gain or loss in yield per acre produced by fertilizer treatment of Italian ryegrass, bottom-land mixture, and white clover cut at 14-day intervals from fertilized and unfertilized plots

Date of cutting	Italian ryegrass			Bottom-land mixture			White clover			
	Ferti- lized	Unfer- tilized	Gain or loss	Ferti- lized	Unfer- tilized	Gain or loss	Date of cutting	Ferti- lized	Unfer- tilized	Gain or loss
May 26.....	1,080.6	703.0	377.6	1,093.1	820.3	272.8	June 2	1,218.3	789.7	428.6
June 9.....	1,472.6	973.8	498.8	1,595.7	1,398.9	196.8	June 16	879.0	794.0	85.0
June 23.....	849.3	677.2	172.1	1,029.5	908.0	121.5	June 30	1,145.3	779.3	366.0
July 7.....	878.6	677.8	100.8	907.6	899.7	7.9	July 14	733.0	598.1	134.9
July 21.....	674.5	775.6	-101.1	656.8	678.7	-21.9	July 28	628.3	570.6	58.7
Aug. 4.....	542.3	359.5	182.8	482.6	374.5	108.1	Aug. 11	666.0	566.5	99.5
Aug. 18.....	544.0	483.0	61.0	396.3	310.7	85.6	Aug. 25	411.7	393.7	18.0
Sept. 1.....	390.3	281.5	108.8	294.2	229.1	65.1	Sept. 8	304.2	389.3	-85.1
Sept. 15.....	385.0	277.1	107.9	216.5	226.6	-10.1	Sept. 22	330.7	215.2	115.5
Sept. 29.....	323.1	187.8	135.3	239.6	163.5	76.1	Oct. 6	158.4	140.4	18.0
Oct. 13.....	250.0	148.6	101.4	218.7	155.2	63.5	Oct. 20	128.2	77.4	50.8
Oct. 27.....	156.8	144.3	12.5	169.2	131.4	37.8				
Nov. 10.....	124.9	134.6	-9.7	154.0	110.3	43.7				
Nov. 24.....	63.0	58.9	4.1	71.2	56.9	14.3				
Dec. 8.....	26.0	22.8	3.2	32.6	19.9	12.7				
Dec. 22.....	57.8	36.3	21.5	61.2	38.7	22.5				
Total.....	7,818.8	6,041.8	1,777.0	7,618.8	6,522.4	1,096.4		6,604.1	5,314.2	1,289.9

On the white-clover plots, although they were seeded to a pure stand, there was at all times a small percentage of grass present in the clippings. During the late spring and summer months this consisted to a large extent of annual bluegrass (*Poa annua* L.), but during the fall and winter months some velvet grass (*Holcus lanatus* L.) also invaded the clover plots. While the white clover was growing rapidly, the percentage of grass was small, but as the growth of the clover decreased, the grass became more and more apparent, until during November and December the samples from the plots consisted mostly of foreign species. For this reason, the data on white clover for November and December are not presented.

The single fertilizer treatment applied early in the spring was not instrumental in maintaining a uniform production over the growing season. In fact, the high production obtained during the late spring months was still further increased by the fertilizer application. This was to be expected since the fertilizer application contained a considerable quantity of nitrogen. The fertilizer was instrumental in maintaining, with few exceptions, a somewhat higher production than was obtained on the unfertilized plots.

The percentages of dry matter of the different cuts are not presented in full, since it was often necessary to cut the plots when the grass was wet with rain or dew. For this reason, these data are not considered to represent exact values. The total dry weights for the season and the seasonal average percentages of dry matter are presented in Table 3.

TABLE 3.—Total dry weights in pounds per acre of, and average percentages of dry matter in, Italian ryegrass, bottom-land mixture, and white clover harvested from fertilized and unfertilized plots

Treatment	Italian ryegrass		Bottom-land mixture		White clover	
	Dry weight	Dry matter	Dry weight	Dry matter	Dry weight	Dry matter
Fertilized.....	<i>Pounds</i> 7,818.8	<i>Per cent</i> 16.44	<i>Pounds</i> 7,618.8	<i>Per cent</i> 16.10	<i>Pounds</i> 6,604.1	<i>Per cent</i> 13.23
Unfertilized.....	6,041.8	16.72	6,522.4	15.82	5,314.2	14.90

The average percentage<sup>9</sup> (weighted average) of crude protein for the season as presented in Table 4 shows that there has been no great increase in the protein content as a result of a single fertilizer application. The greatest increase for the season, 1.13 per cent, was obtained in the white clover. The bottom-land mixture, on the other hand, showed an actual decrease of 1.05 per cent in protein content on the fertilized plot. It should be noted, however, that there is in all three crops, an increase in the percentage of protein on the fertilized plots for the first two or three months, indicating a possible influence of the nitrogen application. This suggests that frequent applications of nitrogenous fertilizers might result in a materially increased percentage of protein. Enlow and Coleman (3) found this to be the case.

<sup>9</sup> The average percentage for the season of crude protein, etc., was obtained by computing the production for each cutting and comparing the total for the season with the total yield of dry matter.

TABLE 4.—Percentage constitution on a dry-matter basis of Italian ryegrass, bottom-land mixture, and white clover, harvested in different months from fertilized and from unfertilized plots

## CRUDE PROTEIN

Month	Italian ryegrass		Bottom-land mixture		White clover	
	Ferti- lized	Unferti- lized	Ferti- lized	Unferti- lized	Ferti- lized	Unferti- lized
May.....	23.62	22.50	28.58	27.12	-----	-----
June.....	22.98	21.62	26.06	25.62	29.01	27.00
July.....	20.56	21.47	21.98	23.75	30.36	28.81
August.....	21.47	23.03	20.98	28.95	30.20	29.74
September.....	26.14	26.05	25.27	26.67	28.31	30.24
October.....	29.50	28.43	28.55	35.36	33.98	33.04
November.....	31.47	31.86	30.90	25.85	-----	-----
December.....	28.11	30.18	29.29	30.35	-----	-----
Weighted average.....	23.42	23.17	25.23	26.28	29.63	28.50

## CRUDE FIBER

May.....	14.78	14.36	16.26	15.94	-----	-----
June.....	20.75	18.50	19.24	18.79	15.70	14.08
July.....	19.70	19.00	17.97	18.19	14.54	14.20
August.....	20.71	18.69	20.00	17.14	14.87	14.03
September.....	18.46	17.17	18.29	15.86	13.89	13.58
October.....	16.24	15.15	15.70	14.76	12.93	13.82
November.....	15.87	15.17	15.91	15.35	-----	-----
December.....	11.98	12.36	12.26	12.83	-----	-----
Weighted average.....	18.94	17.68	18.18	17.52	15.03	14.03

## ETHER EXTRACT

May.....	3.52	3.03	2.95	2.80	-----	-----
June.....	3.69	3.13	3.52	3.00	3.52	3.49
July.....	3.49	3.52	2.92	3.16	3.82	3.67
August.....	3.70	3.74	2.86	2.82	3.15	3.69
September.....	3.77	3.73	3.86	4.76	7.48	3.77
October.....	3.64	4.85	4.74	4.79	5.49	4.19
November.....	5.45	4.63	5.19	4.99	-----	-----
December.....	5.30	5.29	5.29	4.98	-----	-----
Weighted average.....	3.69	3.53	3.40	3.31	3.99	3.63

## CRUDE ASH

May.....	11.02	9.76	13.30	11.92	-----	-----
June.....	13.00	11.67	13.20	12.39	11.51	10.62
July.....	13.04	12.38	12.16	12.01	10.01	9.34
August.....	13.30	12.92	12.45	11.88	9.18	8.25
September.....	15.97	14.13	13.53	12.53	9.14	11.10
October.....	14.09	13.29	12.40	12.45	10.14	10.55
November.....	12.57	13.39	12.10	12.48	-----	-----
December.....	9.23	16.55	9.30	9.07	-----	-----
Weighted average.....	13.29	12.29	12.83	12.17	10.53	9.96

## NITROGEN-FREE EXTRACT

May.....	47.05	50.35	38.91	42.22	-----	-----
June.....	39.58	45.08	37.98	40.20	40.26	44.81
July.....	43.21	43.63	44.97	42.89	41.27	43.98
August.....	40.82	41.62	43.71	39.21	42.60	44.29
September.....	35.66	38.92	39.05	40.18	41.18	41.31
October.....	36.53	38.28	38.61	32.64	37.46	38.40
November.....	34.64	34.95	35.90	41.33	-----	-----
December.....	45.39	35.62	43.86	42.77	-----	-----
Weighted average.....	40.75	43.33	40.36	40.72	40.82	43.88

TABLE 4.—Percentage constitution on a dry-matter basis of Italian ryegrass, bottom-land mixture, and white clover, harvested in different months from fertilized and from unfertilized plots—Continued

## CALCIUM

Month	Italian ryegrass		Bottom-land mixture		White clover	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
May.....	0.54	0.41	0.69	0.67	-----	-----
June.....	.62	.56	.64	.69	1.32	1.06
July.....	.58	.59	.58	.67	1.22	1.13
August.....	.57	.63	.54	.65	1.02	1.00
September.....	.65	.64	.59	.63	.96	.96
October.....	.64	.50	.51	.56	.84	.76
November.....	.60	.46	.44	.42	-----	-----
December.....	.51	.44	.43	.34	-----	-----
Average for season.....	.596	.561	.602	.655	1.194	1.040

## PHOSPHOROUS

Month	Italian ryegrass		Bottom-land mixture		White clover	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
May.....	0.54	0.47	0.55	0.65	-----	-----
June.....	.64	.57	.63	.60	0.68	0.63
July.....	.60	.60	.51	.52	.49	.50
August.....	.61	.58	.51	.51	.47	.48
September.....	.63	.56	.58	.50	.46	.45
October.....	.65	.60	.54	.60	.52	.52
November.....	.69	.65	.63	.70	-----	-----
December.....	.57	.58	.70	.63	-----	-----
Average for season.....	.612	.569	.570	.569	.575	.548

The increased percentage of protein found in the Italian ryegrass (and to a lesser degree in the bottom-land mixture) during the later months is probably due to a less advanced stage of maturity reached by these crops in the 14-day interval at this period of the year.

Table 4 shows that there is in the Italian ryegrass and bottom-land mixture a considerable decrease in the percentage of crude fiber from August to December. This decrease is undoubtedly due to the fact that the grass, although cut at equal intervals throughout the season, was actually cut at a more immature stage in the later months than in the months of rapid growth. The fact that the Italian ryegrass, during the period of rapid growth, often showed seed heads before the end of the 14-day period, supports this view.

The data show that there has been a rather consistent increase in the percentage of fiber in the fertilized crops over that in the unfertilized crops. A more advanced stage of maturity attained in the 14-day period on the fertilized plots may be responsible for this.

Taking the average for the whole season, there has been a slight increase in the ether extract content of the pasture crops as a result of the fertilizer treatment. (Table 4.) This increase is in no case large but is found in all three crops. (This is in agreement with the results obtained by Archibald and Nelson (1), who found an increase in the percentage of ether extract as a result of fertilization.)

There are, however, a number of cases where the percentage of ether extract in the unfertilized crops is greater than that in the fertilized crops. For this reason the comparatively insignificant increases for the season shown in Table 4 can not be considered conclusive.

In all three crops, there is an increase in the percentage of ether extract as the season advances.

The figures in Table 4 show an appreciable increase in the percentage of crude ash on all fertilized plots for the season. These figures, however, represent the percentage of crude ash rather than silica-free ash.

The percentages of nitrogen-free extract of the different crops are presented in Table 4 only for comparison. While appreciable differences exist between the fertilized and unfertilized crops, these differences are not considered significant, since the data were secured by subtraction.

Table 4 indicates that no significant changes in calcium content have occurred as a result of the fertilizer treatment except in the white clover during June and July. The greatest difference lies between the different crops rather than in seasonal differences in a single crop, the white clover having a decidedly higher calcium content than the Italian ryegrass. The bottom-land mixture ranks between the other two grasses in calcium content, showing the effect of the clover in the mixture.

There is a decided drop in the calcium content of the white clover during the fall months, and the bottom-land mixture shows a similar decline. While this is apparently a seasonal change in the calcium content of the crop, it is probably due, at least in part, to the gradual decrease in the proportion of clover on these plots, and the resulting increase in the proportion of grass (by invasion on the clover plots). From the data shown it would seem that the percentage of calcium could be increased most readily by increasing the percentage of clover in a pasture crop.

An indication as to the amount of total calcium contained in soil of the type used in this experiment is found in the work of Holtz (6) in 1930. Holtz found an average of 1.855 per cent total calcium in the soils of the Puget series. Two samples of Puget sandy loam obtained from the western Washington station average 2.21 per cent total calcium.

The percentage of phosphorus recovered in the pasture cuttings, under the conditions of the experiment, seems to be even less affected than the percentage of calcium by fertilizer applications or seasonal changes in production. While variations occur between different cuttings, significant differences are not apparent, either between fertilized and unfertilized plots, or between different crops. If any difference exists it is shown in the white clover and bottom-land mixture by the slight lowering of the phosphorus content during the dry summer months. The percentages of phosphorus, however, are in all cases comparatively high, as a comparison with the results obtained by other investigators will show. The percentages of phosphorus found in pasture grass by various investigators are:

Investigator	Percentage of phosphorus
Archibald and Nelson (1)-----	0.28 to 0.33.
Ellenberger, Newlander, and Jones (2)-----	0.32 average.
Forbes, Whittier, and Collison (4)-----	0.168 to 0.349 (1908-1909).
Rigg and Askew (9) (from pastures giving good results with livestock).	0.39 to 0.56 (0.90 to 1.29 $P_2O_5$ ).
Woodman, Bee, and Griffith (11) (dried-grass cakes).	0.39 to 0.43 (0.90 to 1.00 $P_2O_5$ ).
Woodman and Evans (13) (mineral-deficient forage).	0.09 to 0.11 (0.21 to 0.27 $P_2O_5$ ).
Western Washington Station-----	0.45 to 0.70.

Holtz (6) found that soils of the Puget series averaged 0.0285 per cent available phosphorus. Two samples of Puget sandy loam from the western Washington station averaged 0.0444 per cent available phosphorus. This high percentage of available phosphorus probably accounts for the high percentage of phosphorus found in the crops studied, and the failure of the phosphorus application to increase the percentage of phosphorus in the herbage.

The figures showing the quantities of phosphorus per acre recovered from the different plots are quite striking. These figures are presented in Table 5.

TABLE 5.—*Phosphorus (pounds per acre) found in Italian ryegrass, bottom-land mixture, and white clover, from fertilized and unfertilized plots, as compared with the quantity applied in fertilizer*

Crop	Treatment	Yield of dry matter, per acre	Phosphorus in dry matter	Phosphorus contained in crops	Phosphorus per acre applied in fertilizer	Phosphorus (as $P_2O_5$ ) contained in crops
		<i>Pounds</i>	<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Italian ryegrass.....	Fertilized.....	7,818.8	0.612	47.88	38.23	109.59
	Unfertilized.....	6,041.8	.569	34.41	-----	78.76
Bottom-land mixture.....	Fertilized.....	7,618.8	.570	43.45	38.23	99.45
	Unfertilized.....	6,522.4	.569	37.14	-----	85.01
White clover.....	Fertilized.....	6,604.1	.575	37.97	38.23	86.91
	Unfertilized.....	5,314.2	.548	29.14	-----	66.07

On the fertilized plots (except the white clover) more phosphorus was taken from the soil than was applied to the soil in the fertilizer. Even on the unfertilized plots, the phosphorus taken up by the crop was equivalent to a heavy application of phosphate fertilizer. While under actual pasture conditions a certain amount of phosphorus is returned to the soil in the manure, it is evident that under conditions of heavy yield, the pasture crop makes a heavy drain on the phosphorus resources of the soil.

The figures in Table 6 show the influence of clover on the calcium-phosphorus ratio. Under the conditions of the experiment, the presence or absence of clover has apparently had a much greater influence on the relative amounts of calcium and phosphorus in the dry matter than had the fertilizer treatment.

TABLE 6.—*Calcium-phosphorus ratios of dry matter of pasture crops cut at 14-day intervals from fertilized and from unfertilized plots*

Treatment	Italian ryegrass	Bottom-land mixture	White clover
Fertilized.....	1:1.014	1:0.94	1:0.48
Unfertilized.....	1:1.026	1: .86	1: .52

The data in Table 7 show that the yield of crude protein on the fertilized plots was generally higher than the yield on the unfertilized plots. This increase was due, however, largely to the increased yield of dry matter on the fertilized plots rather than to an increased percentage of protein in the dry matter. The largest increase in the percentage of protein due to fertilization (1.13 per cent in the white

clover) is comparatively insignificant. It is evident, therefore, that the increase in the yield of dry matter as a result of a fertilizer application, is a more important factor than an increase in the protein content. This is especially true since the nutritive ratio of immature grass is already comparatively narrow, as shown by the work of Woodman, Blunt, and Stewart (12) and Ellenberger, Newlander, and Jones (2).

TABLE 7.—Yield of crude protein in pounds per acre, and gain or loss in yield produced by fertilizer treatment of Italian ryegrass, bottom-land mixture, and white clover cut at 14-day intervals from fertilized and unfertilized plots

Date of cutting	Italian ryegrass			Bottom-land mixture			White clover			
	Fertilized	Unfertilized	Gain or loss	Fertilized	Unfertilized	Gain or loss	Date of cutting	Fertilized	Unfertilized	Gain or loss
May 26.....	255.23	158.17	97.06	312.40	222.46	89.94	June 2	353.42	213.21	140.21
June 9.....	338.40	210.53	127.87	415.83	358.39	57.44	June 16	254.99	214.38	40.61
June 23.....	155.16	146.41	48.75	268.28	232.62	35.66	June 30	332.25	210.41	121.84
July 7.....	180.64	166.99	13.65	199.49	213.07	-14.18	July 14	222.53	172.31	50.22
July 21.....	138.67	166.52	-27.85	144.36	161.19	-16.83	July 28	191.05	164.38	26.67
Aug. 4.....	116.43	82.79	33.64	101.24	108.41	-7.17	Aug. 11	201.13	168.47	32.66
Aug. 18.....	116.79	111.23	5.56	83.14	89.94	-6.80	Aug. 25	124.33	117.08	7.25
Sept. 1.....	102.02	73.33	28.69	74.34	61.10	13.24	Sept. 8	86.11	117.72	-31.61
Sept. 15.....	100.63	72.18	28.45	54.70	60.43	-5.73	Sept. 22	93.62	65.07	28.55
Sept. 29.....	84.45	48.92	35.53	60.54	43.60	16.94	Oct. 6	53.82	46.38	7.44
Oct. 13.....	73.75	42.24	31.51	62.43	54.87	7.56	Oct. 20	43.56	25.57	17.99
Oct. 27.....	46.25	41.02	5.23	48.30	46.46	1.84	-----	-----	-----	-----
Nov. 10.....	39.30	42.88	-3.58	47.58	28.51	19.07	-----	-----	-----	-----
Nov. 24.....	19.82	18.76	1.06	22.00	14.70	7.30	-----	-----	-----	-----
Dec. 8.....	7.30	6.88	0.42	9.54	6.03	3.51	-----	-----	-----	-----
Dec. 22.....	16.24	10.95	5.29	17.92	11.74	6.18	-----	-----	-----	-----
Total.....	1,831.08	1,399.80	431.28	1,922.09	1,714.12	207.97	-----	1,956.81	1,514.98	441.83

In comparing the quantity of nitrogen actually applied on the fertilized plots with the excess of nitrogen (over the unfertilized crops) recovered in the dry matter of the fertilized crops (Table 8), it is interesting to note that in the Italian ryegrass and white clover, the amount of this excess nitrogen is actually much greater than the amount of nitrogen applied in the fertilizer.

TABLE 8.—Excess nitrogen (pounds per acre) in Italian ryegrass, bottom-land mixture, and white clover, from fertilized plots, (over unfertilized plots) as compared with the quantity applied in fertilizer

Crop	Excess protein (over unfertilized crops) contained in fertilized crops	Excess nitrogen (over unfertilized crops) contained in fertilized crops	Nitrogen applied per acre	Excess nitrogen contained in fertilized crop (over unfertilized crop) expressed as percentage of nitrogen applied as fertilizer
Italian ryegrass.....	431.28	69.00	45	153.33
Bottom-land mixture.....	207.97	33.27	45	73.93
White clover.....	441.83	70.69	45	157.09



## SUMMARY

Studies on the yield of Italian ryegrass, bottom-land mixture, and white clover during the 1930 season made by cutting plots of these crops at regular intervals of 14 days, show that a period of heavy production during May and June was followed by a constant drop in the production for the remainder of the season.

The total yields of dry matter in pounds per acre for the season of the different crops are as follows: Italian ryegrass, fertilized, 7,818.8; unfertilized, 6,041.8; bottom-land mixture, fertilized, 7,618.8; unfertilized 6,522.4; and white clover, fertilized 6,604.1; unfertilized 5,314.2.

The percentages of crude protein for the season of the different crops are: Italian ryegrass, fertilized, 23.42; unfertilized, 23.17; bottom-land mixture, fertilized, 25.23, unfertilized, 26.28; and white clover, fertilized, 29.63, unfertilized 28.50.

The yields of dry matter and crude protein are, with few exceptions, higher through the season in the fertilized crops.

A high percentage of crude protein is maintained throughout the season by cutting pasture crops at 14-day intervals. The percentage of crude protein shows a tendency to increase during the fall months. This is believed to be due to greater immaturity of the crop at the time of cutting.

Under the conditions of low rainfall occurring in 1930 the presence or absence of clover exerts a greater influence on the protein content than does a single application of fertilizer made at the beginning of the season.

The fiber content of the fertilized crops is consistently higher than that of the unfertilized crops.

The increase in the yield of protein as a result of the fertilizer treatment is 431.28 pounds per acre for the Italian ryegrass, 207.97 pounds for the bottom-land mixture, and 441.83 pounds for the white clover. The use of fertilizer containing 45 pounds of nitrogen per acre resulted in an increased recovery of nitrogen, amounting to 69.00, 33.27, and 70.69 pounds for the Italian ryegrass, bottom-land mixture, and white clover, respectively. As compared with the nitrogen applied, the increases in nitrogen recovery resulting from the fertilizer application amount to 153.33, 73.93, and 157.09 per cent, respectively, for the three crops.

The percentage of ether extract and crude ash are higher in the fertilized crops than in the unfertilized crops. The percentage of ether extract shows a considerable tendency to increase during the fall months.

No marked changes in calcium content occur as a result of the fertilizer treatment. The clover contains by far the highest percentage of calcium, while the bottom-land mixture is intermediate between Italian ryegrass and white clover in this respect. Clover appears to be a more important factor in determining calcium content of a forage crop than does the fertilizer.

The calcium-phosphorus ratio appears to be influenced by the presence or absence of clover. The influence of the fertilizer on the calcium-phosphorus ratio is insignificant. The recovery of phosphorus in high-yielding pasture crops is equivalent to a heavy application of phosphate fertilizer.

Under the conditions of the experiment, the increase in the yield of dry matter is found to be a more important result of the fertilizer treatment than is any increase in the percentage of protein, calcium, or phosphorus.

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COMPOSITION OF RHUBARB AT DIFFERENT STAGES OF MATURITY IN RELATION TO ITS USE IN COOKING AND CANNING<sup>1</sup>

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## REVIEW OF LITERATURE

There is considerable literature dealing with the composition of different portions of the rhubarb plant. Much of this concerns the chemistry of the root and the isolation and identification of the constituents responsible for its therapeutic effects. In the present investigation the composition of the root is of interest because limited quantities of the therapeutically active substances may be present in the petioles and practically all the food constituents of the petiole occur to some extent in the root. The literature on the root is summarized in the standard text books of pharmacology, but particular mention may be made of the work of Tutin and Clewer (32),<sup>3</sup> who identified many of the constituents of the root and cleared up much of the confusion in the terminology of this subject. Of special importance also is the work of Müller (22), who has isolated certain substances occurring in the root.

Because of the possibility that oxalic acid may occur in quantities large enough to be toxic, considerable work has been done on the nature and amounts of the organic acids of the rhubarb plant and on the toxicity of the oxalic acid as well as that of different parts of the plant itself. The results of these investigations (1, 2, 3, 6, 10, 16, 19, 21, 23, 24, 27, 28, 31) show that malic acid predominates in the petiole and that citric acid and oxalic acid occur in much smaller quantities. The total acidity appears to range from 1 to 2 per cent. It is also shown that generally there is not sufficient oxalic acid in the petioles, or leafstalks, to cause harmful effects if they alone are eaten, but most investigators consider it unsafe to eat the leaves when cooked in the same way as spinach or other greens.

From the reports of several investigators (5, 7, 30) on the food constituents of rhubarb, it is evident that the food value is rather low and that the constituents vary considerably.

Several investigators have given attention to the ash constituents of rhubarb. From the work of Sherman (30) it appears that with the exception of potassium the mineral constituents are low.

The vitamin content of rhubarb has been studied by Pierson and Dutcher (26) and by Hessler and Williams (15). Their papers are of interest in the present investigation because they deal to some extent with the methods of preparation and preservation.

<sup>1</sup> Received for publication May 3, 1932; issued April, 1933.

<sup>2</sup> The material used in this investigation in 1928 was kindly furnished by D. N. Shoemaker, of the Division of Horticultural Crops and Diseases.

<sup>3</sup> Reference is made by number (*italic*) to Literature Cited, p. 400.

Cruess (9) states that rhubarb when canned has an intensely corrosive action upon the tin container and causes swelling and sometimes even perforations. The reports of other investigators (8, 17, 18, 29) show a lack of agreement, which may be partly due to differences in the material used.

#### OBJECT OF THE INVESTIGATION

Investigators have found considerable variation in the composition of rhubarb, in its properties when cooked, and in its behavior when canned. The main object of the present investigation was to determine the effect of age on the composition of rhubarb and the relation of its composition to its suitability for cooking and canning. It was thought advisable to determine also whether seasonal conditions affect its composition. The practical canning and cooking tests were accompanied by a study of the biochemistry of the development of the rhubarb petiole and leaf blade and its relation to seasonal conditions. The results of this phase of the investigation are reported in detail elsewhere (10).

#### SOURCE OF MATERIAL

The material used in the canning and cooking tests in 1926 and 1927 was purchased in the market at Washington, D. C. The appearance of the material indicated that it was all of the same variety, either Victoria or a strain closely resembling it. The material was fresh and at a stage of maturity typical of that generally offered for sale in the markets. The leafstalks were 15 to 18 inches long and weighed 60 to 80 g each.

The material used in 1928 was grown in the test plots at the Arlington Experiment Farm, Rosslyn, Va. The variety was Ruby, a seedling of Victoria (*Rheum hybridum* Murr.), introduced by the Central Experimental Farm, Ottawa, Canada, in 1923 (20). This variety was recommended because of the freedom of its leafstalks from stringiness and extreme acidity. The leafstalks are not particularly large but are very numerous and generally contain more than the usual quantity of the red anthocyan pigment in the epidermis. The divisions were obtained from 2-year-old crowns grown in the immediate vicinity of the present planting, which was made in the spring of 1927, the method used being that ordinarily employed in propagating rhubarb. All the chemical analyses reported in this paper were made upon the material grown on the Arlington Experiment Farm in 1928.

#### SOIL AND CULTURE

The soil on which the rhubarb was grown was a moderately fertile loam to which had been added generous quantities of well-rotted manure. In the fall of 1927 the plants were mulched with barnyard manure, but in 1928 no fertilizer or manure was applied until after the last samples were taken for analysis. The plants were given frequent hoeing throughout the year to remove weeds.

#### METHODS OF SAMPLING AND ANALYSIS

Four series of samples of known age were secured in the season of 1928. The leaves were just beginning to make their appearance

aboveground on April 10. On April 21 the petioles were 2 to 4 inches long and the leaves in many cases had not completely unfolded. On this date a large number of petioles were marked by means of tags, so that leaves of known age could be collected at any time desired. Six collections made at intervals of 7 to 12 days and extending up to June 15 constitute the first series. While this series was being taken the plot was gone over at intervals of 3 to 6 days and the new leaves were tagged as the petioles became 2 to 4 inches long; this treatment was continued to October 9. The leaves tagged in April were considered to be about 10 days old; those tagged in June and July about 5 days old. The higher temperatures of June and July caused more rapid development, so that samples of a given age taken in June or July were at a somewhat later stage of growth than those of like age taken in April or May. From the various lots of tagged leaves, three later series, each consisting of petioles 5 to 60 days old, were collected on June 5, July 6, and October 17, respectively. Broken or injured leaves were always discarded. The leaves were picked by pulling them from the crown, as is customary in harvesting rhubarb. The material for analysis was collected about 10 a. m., and 15 to 25 leaves were used to obtain each chemical sample. Thin cross sections were cut from near the middle of the petioles, and from these petioles 100-g samples were weighed out; enough 95 per cent alcohol was added to bring the concentration up to 75 to 80 per cent. The material was brought to boiling and sealed. For total nitrogen determinations similar samples were dried in a vacuum oven at 70° to 75° C.

The preserved samples were extracted with alcohol and made up to volume. Aliquot portions of these were used for the various determinations. The acid was titrated with N/10 NaOH and calculated as malic acid. The sugars were determined by the volumetric permanganate method recommended by the Association of Official Agricultural Chemists (4). The acid-hydrolyzable polysaccharides were determined upon the residue after extraction with alcohol. Total nitrogen was determined by the Kjeldahl method as modified to include nitrate nitrogen. Nitrate nitrogen was determined by the ferrous chloride method as recommended in the Methods of the Association of Official Agricultural Chemists (4) for nitrates in meats. The total astringency was determined by the Proctor-Loewenthal method (25).

#### ANALYTICAL RESULTS

The first series differed from the others in that the successive samples making it up were taken at 10-day to 12-day intervals over a period of 55 days, hence under environmental conditions that differed from sample to sample. Later, all the samples constituting a series were taken at one time, and hence had been subjected to identical conditions. Allowing for these differences, the general course of chemical change during development was, broadly speaking, the same in the four series, as is indicated by the similarity of the curves shown in Figures 1 to 6 and by the data given in Table 1.

TABLE 1.—*The composition of rhubarb petioles at different stages of development expressed in percentage of fresh green weight*

SERIES A												
Date of sampling	Age	Length of petiole	Total solids	Solids soluble in alcohol	Solids insoluble in alcohol	Total sugars	Acid-hydrolyzable polysaccharides	Acids	Tannins	Nitrate nitrogen	Amino nitrogen	Total nitrogen
	Days	Inches	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
April 21.....	10	2-4	5.21	2.96	2.25	0.65	0.55	0.999	0.096	0.014	0.045	0.211
May 5.....	24	10-12	4.80	2.44	2.36	.30	.53	.948	.058	.030	.029	.203
May 19.....	38	12-14	5.71	2.80	2.91	.42	.67	.884	.086	.067	.027	.178
May 26.....	45	14-18	5.87	2.90	2.97	.60	.68	1.000	.109	.065	.036	.155
June 5.....	55	14-18	5.81	2.96	2.85	.67	.58	.818	.073	.077	.024	.127
June 15.....	65	14-18	6.52	2.96	3.56	.66	.70	.749	.110	.100	.020	.130
SERIES B												
June 5.....	5	3-4	5.33	2.84	2.49	0.40	0.54	1.080	0.122	0.024	0.043	0.198
	8	6-10	4.83	2.60	2.23	.39	.48	1.110	.109	.029	.045	.160
	11	10-12	4.60	2.48	2.12	.33	.46	1.080	.127	.030	.055	.136
	15	12-14	4.84	2.72	2.12	.39	.47	1.110	.096	.032	.040	.142
	19	14-16	4.56	2.56	2.00	.29	.42	1.070	.088	.035	.039	.149
	25	16-18	5.25	2.96	2.28	.52	.45	1.085	.091	.038	.030	.127
	32	18-21	5.81	3.16	2.65	.60	.58	1.140	.087	.044	.030	.134
	40	18-21	6.17	3.37	2.80	.90	.72	1.160	.079	.056	.026	.130
55	14-18	5.81	2.96	2.85	.67	.58	.818	.073	.077	.024	.127	
SERIES C												
July 6.....	5	4-16	6.57	3.44	3.13	0.40	0.76	1.520	0.168	0.017	0.071	0.267
	9	10-12	6.45	3.44	3.01	.50	.79	1.580	.153	.022	.068	.228
	13	12-16	6.15	3.20	2.95	.60	.77	1.380	.123	.026	.057	.197
	28	18-24	7.19	3.60	3.59	1.05	.84	1.320	.201	.027	.043	.149
	42	20-28	7.43	3.48	3.95	.91	.78	1.150	.162	.053	.038	.148
60	20-28	6.82	3.96	3.86	.56	.75	.781	.265	.073	.010	.121	
SERIES D												
October 17.....	8	9-14	6.29	3.56	2.73	1.04	0.63	1.560	0.111	0.023	0.070	0.201
	12	14-18	6.35	3.44	2.91	1.04	.64	1.430	.114	.026	.070	.186
	24	16-20	7.22	3.76	3.46	1.14	.74	1.300	.110	.026	.050	.180
	40	16-20	7.23	3.84	3.39	1.05	.72	1.280	.115	.030	.041	.159
	60	16-20	5.64	2.52	3.12	.58	.67	.934	.100	.045	.039	.132

## SOLIDS

Table 1 shows the results of analyses expressed in percentages of the fresh green weight. A high moisture content for all the samples is shown by the low percentages of total solids, although these varied somewhat with the season in which the samples were taken. Early in the season the solids were below 5 per cent in some samples, whereas later in the season they were above 7 per cent in some samples. The curves for the total dry matter in the four series of samples (fig. 1) show that the total solids were generally lower in the series taken in April and June than in the series taken in July and October. The weather records show that the samples taken in April and May grew under conditions of shorter days, and hence with less sunshine and with a lower temperature. The relative intensity of the photosynthetic activity may account for some of the differences in the four series. Since the samples were taken in July and October after several

days in which no rain fell it might be assumed that the soil moisture had a considerable effect. These data indicate that a high percentage of solids may be expected in plants grown under conditions of low or medium soil moisture, high temperature, and long hours of sunshine.

The data in Table 1 show also that the percentage of solids varied somewhat with age. Very young petioles, 3 to 4 inches long, had a medium percentage of solids; this decreased during the next few days, then increased, and finally, with one exception, decreased in the very old samples. The petioles seem to have been at about their lowest in solids at the time they were picked for market, or a little earlier.

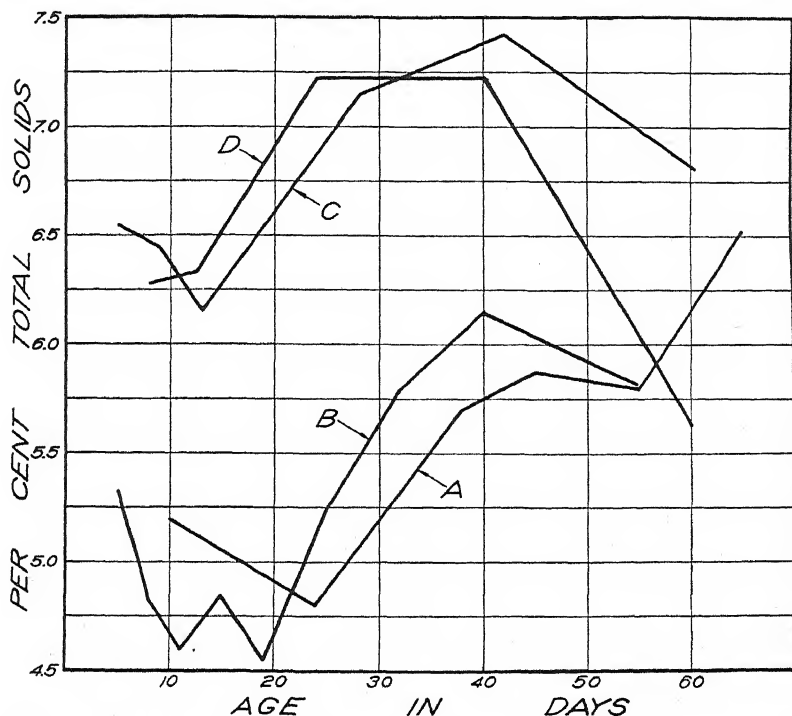


FIGURE 1.—Changes in total solids of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

About half the total solids were soluble and half insoluble in alcohol. Both soluble and insoluble solids were higher in the series taken in April and May. Insoluble solids varied with age, being lowest in the young samples and highest in the old ones. The soluble solids increased with age or remained nearly constant.

#### SUGARS

The total sugars were very low and showed no very significant change with age. However, the series taken on July 6 and on October 17 were slightly higher in sugars than those taken earlier. Figure 2 shows the curves for total sugars.

## ACID-HYDROLYZABLE POLYSACCHARIDES

The acid-hydrolyzable polysaccharides were also very low. The material in these samples gave a negative iodine test for starch. Differences due to age are not very significant.

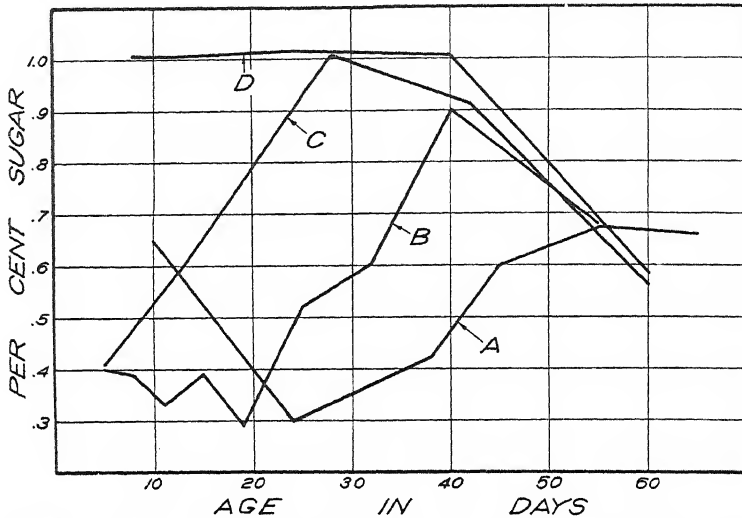


FIGURE 2.—Changes in total sugar content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

## ACIDS

One of the chief characteristics of rhubarb as a food material is its high acidity. In the variety studied, which is considered comparatively low or medium in acidity, the acid content calculated as malic acid on the fresh green-weight basis may be as high as 1.5 per cent. The high acid content, together with the very low sugar content, makes this variety extremely tart. Except in very old samples, the acidity is considerably higher in the series taken in July and October than in the series taken in April and May. The climatic conditions favorable to high acidity seem to be long hours of intense sunshine, medium or low rainfall, and high temperature. Rhubarb is often said to vary greatly in acidity. In many cases this may be due to varietal differences, but the present study shows that it may also be due to differences in stage of maturity or climatic conditions. The tendency already noted of the titratable acidity to decrease with age is not very noticeable until after the rhubarb has passed the stage in which it is in prime condition for table use. In very old samples the decrease is very marked. It is apparent from Figure 3 that the acidity may remain nearly constant during most of the period in which the material is in prime condition for table use.



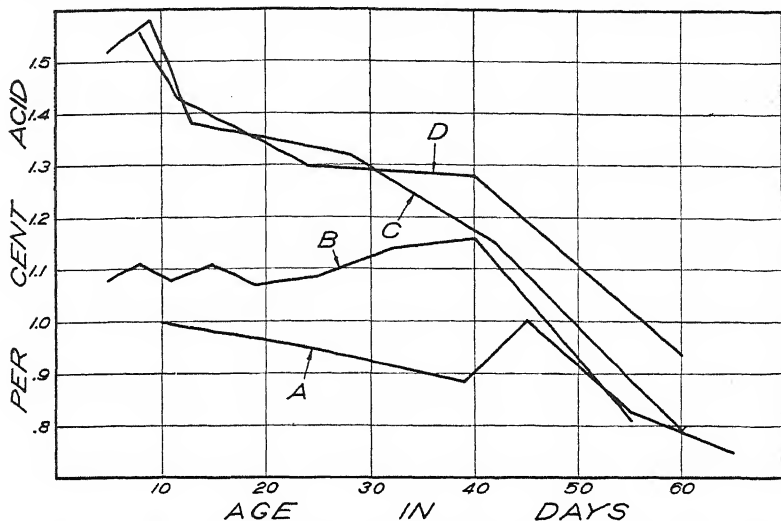


FIGURE 3.—Changes in titratable acidity of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

#### TOTAL NITROGEN

The total nitrogen in rhubarb is decidedly low, as it is in many fruits. The nitrogen content of the samples analyzed varied from 0.267 to 0.121 per cent. (Table 1.) If these percentages are multiplied by 6.25, the factor used for computing the percentage of protein,

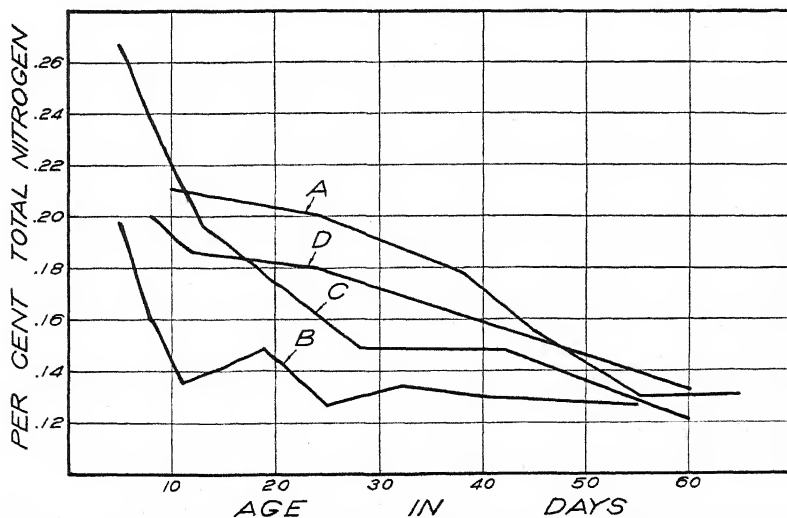


FIGURE 4.—Changes in total nitrogen content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

it will be apparent that the protein content of rhubarb is often less than 1 per cent. The total nitrogen, highest in the young samples, decreases rapidly as the petiole develops and more slowly in the older stages. (Fig. 4.)

## NITRATE NITROGEN

Changes in the content of nitrate nitrogen are of particular interest. Table 1 shows that in certain cases a high percentage of the total nitrogen is in the form of nitrates. The nitrate nitrogen was lowest in the young samples and increased steadily throughout the life of the leaf. (Fig. 5.) In one sample 77 per cent of the total nitrogen of the old petioles was in the form of nitrates. It may be inferred that the presence of such a high percentage of nitrate is significant as a factor in the corrosion of the metal of the tin container.

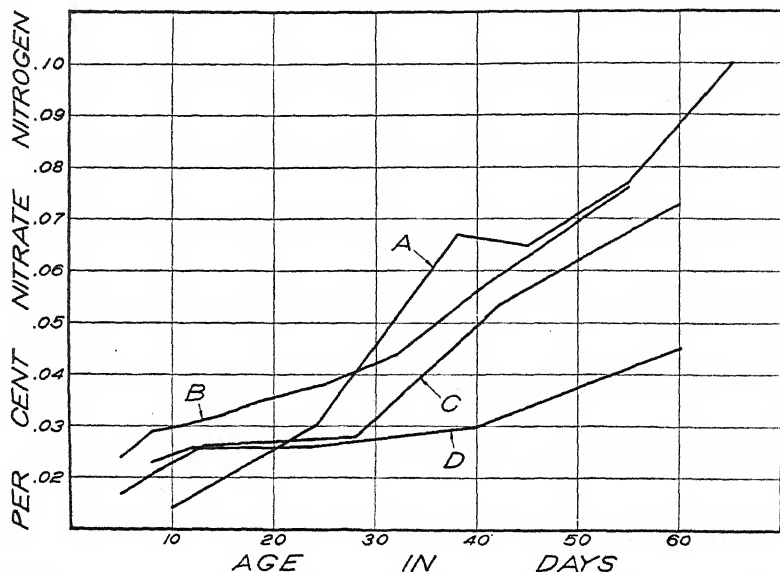


FIGURE 5.—Changes in nitrate nitrogen content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

The series of samples taken in October was lower in nitrate nitrogen than those taken earlier. It seems probable that this was due to the reduction of available nitrates in the soil as the season advanced and that the variations in the different series were directly related to variations in the available nitrates in the soil. It would therefore be expected that soil conditions and fertilizer treatment would very materially affect the amount of nitrate nitrogen.

## AMINO NITROGEN

In many cases a considerable portion of the total nitrogen is amino nitrogen. It was highest in the young material and decreased as the petiole grew older. Figure 6 shows the results for the four series of samples.

## TANNINS

Rhubarb contains a small percentage of astringent substances, or tannins, and it is probable that these are partly responsible for its characteristic flavor. It is not clear just what factors caused the variations of the tannin content of the samples shown in Table 1.

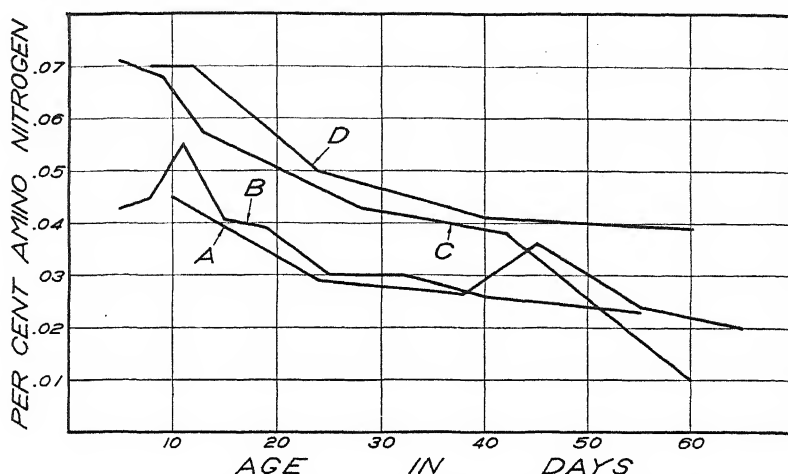


FIGURE 6.—Changes in amino nitrogen content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

## COOKING TESTS

### MATERIAL AND METHODS

Rhubarb is commonly used early in the spring, but cooking tests indicate that it may be used at any time throughout the season. In view of the variations in composition as shown by the chemical analyses, some difference in quality from time to time should be expected. The material may be used at any age up to about 30 days when development occurs in the very hottest part of the season, or up to 50 days when development occurs in the cooler part of the season. It becomes somewhat stringy at a much earlier stage in the middle of the summer than in the early spring.

From the standpoint of yield it is not advantageous to harvest when the petioles are only 3 to 4 inches long. The present practice of picking the leaves when the petioles have almost reached their maximum length seems to be about the best procedure. Rhubarb has very nearly maximum moisture content at this stage, yet the difference from earlier stages is not great and may not be very important.

In making the cooking tests some samples were taken in which the petioles were 10 days old and 3 to 4 inches long and, for comparison, other samples were taken that were 10, 18, 30, and 40 days older than the first. The petioles were cut into pieces one-half inch long, placed in a kettle with a small amount of cold water, and allowed to boil for 10 minutes. Then 30 per cent by weight of sugar was added and the material was stirred until the sugar was dissolved. In these tests the very young samples were considered superior to all others. They seemed slightly milder and of better consistency than any of the older ones. There seemed to be very little difference between samples that were taken 18 days and 30 days after the first samples, indicating that the quality remains nearly constant for a considerable time after the petiole ceases its rapid rate of growth. The older samples seemed somewhat more fibrous and less desirable than those taken 18 days

after the first samples. The increase in alcohol-insoluble residue shown by the analytical results indicates that the fiber increases; this is in accordance with the cooking tests.

#### PECTIN CONTENT

When cooked, rhubarb softens and becomes a viscous, semiliquid, colloidal mass. When tested for starch the result was negative. When strained through cheesecloth the liquid gave a voluminous precipitate with alcohol. A precipitate was also formed with calcium hydroxide. When treated with ammonia and then neutralized, an almost insoluble precipitate was formed. When the very young sample was ground and the juice pressed out without heating, a precipitate was formed with alcohol; but the juice of the older petioles gave very little precipitate when so treated. These tests indicate that there is considerable material of the pectin group in the petiole of the rhubarb plant. A large part seems to be protopectin; pectin and pectic acid are probably present also. While pectin was not determined quantitatively, the difference between the precipitates with alcohol indicated that the protopectin decreased greatly as the petiole became older.

#### ADJUSTMENT OF THE SUGAR-ACID RATIO AND THE CONSISTENCY

The low sugar content and the high acid content of rhubarb make it necessary to add sugar in order to obtain an agreeable taste. The acidity is sometimes so high that the material is improved by soaking in warm water for a short time or in cold water for a long time. For the same reason, partial neutralization is sometimes recommended; a milder product is thus obtained, which will require less sugar to sweeten properly. The acid content, which has been shown to vary considerably with the season, will influence the quantity of sugar that must be added. In these tests, the addition of 30 to 40 per cent of sugar to material having an acid content of 1 per cent made a sauce agreeable to the writers. Of course, individuals vary greatly in their preference as to sweetness. However, the writers concluded that a sugar ratio of about 1:3 is agreeable to most individuals.

Despite the pectin content the moisture content of rhubarb is so high that in making pies it is usually found desirable to add flour or cornstarch to give the material the proper consistency.

#### CANNING TESTS

In 1926 and 1927 canning tests were made with rhubarb purchased in the market at Washington, D. C. In 1928 the rhubarb used was grown on the Arlington Experiment Farm, as described earlier in this paper. The age of the material in 1926 and 1927 was not known, but it appeared to be in prime condition for table use. In 1928 the rhubarb collected was 20 to 30 days old. The material was brought to the laboratory, trimmed, washed, cut into pieces one-half inch long, and packed in both tin and glass containers as follows:

- (1) The pieces were packed tightly in cans or jars, and water was added to fill the interspaces; the containers were then exhausted three minutes in steam at 100° C. and sealed.
- (2) The treatment was the same as in 1 except that 60 per cent sirup was added instead of water.
- (3) The treatment was the same as in 1 except that no exhaust was given.

(4) The treatment was the same as in 1, except that 60 per cent sirup was added instead of water and no exhaust was given.

(5) The pieces were steamed for five minutes at 100° C. and while hot were pressed tightly into the cans without the addition of either sugar or water.

(6) The treatment was the same as in 5, except that 40 per cent by weight of sugar was added to the hot material and stirred until dissolved.

Some of the rhubarb receiving each treatment was packed in weighed No. 2 cans of three types, namely, plain tin, single-enamel coke-plate, and reenamed charcoal-plate cans. Additional lots of rhubarb receiving treatments 1 and 3 were packed in No. 3 plain tin and single-enamel coke-plate cans. Additional lots receiving treatments 1, 5, and 6 were packed in quart glass jars.

The cans were processed at 100° C. for 15 minutes and then stored for one year. The tin cans were weighed before they were packed with the material and again when they were opened and examined. Large quantities of yellowish crystals or incrustations that had formed in the can during storage frequently adhered tenaciously. This made the method inaccurate for measuring the total amount of corrosion. It was obvious, however, from the differences in the weight of the cans at the beginning and at the end of the test, that unusually large amounts of tin and iron were dissolved from the cans by the material packed in them.

When examined one year after storage the quality of the rhubarb in the glass jars was very nearly equal to that of rhubarb prepared immediately from fresh material.

All the plain tin cans processed without exhaust (treatments 3 and 4) were perceptibly swelled; some were flippers (slightly swelled), and others were swelled tightly as a result of hydrogen formation. The gas had no foul odor and burned violently when ignited, indicating that it was hydrogen. The cans were severely corroded and frequently weighed 0.5 to 1 g less than when the material was placed in them. The tin coating seemed to have been entirely removed from the iron, which appeared dark in color and in many cases was partly covered with adhering crystals or a yellowish incrustation that could not be readily removed by washing. The rhubarb appeared nearly normal, except that it was light brown in color and dull in appearance as compared with that canned in glass, and had a rather disagreeable, metallic taste. It was on the whole an unsatisfactory product. None of the cans were perforated.

The plain tin cans which were sealed hot (treatments 1, 2, 5, and 6) were frequently slightly swelled, but in many cases were entirely normal in appearance. The tin seemed to have been entirely removed from the interior of the can and it appeared almost as severely corroded as the unexhausted cans. None of the cans had become perforated. The contents of the cans, in every way similar to those of the unexhausted cans, were still distinctly acid, having a pH value of 3.52 to 4.0 (0.0001 to 0.0003 N). Since the iron was apparently exposed, the corrosion might be expected to proceed at a rapid rate; however, it was apparent that the action at the end of one year was very much slowed down if not entirely stopped. This was believed to be due to the formation of protective films or incrustations on the surface of the iron. Yellowish coatings, which probably consisted of crystalline or colloidal iron oxalate, were frequently observed.

All the single-enamel cans showed less gas formation than the plain tin cans and were frequently normal in appearance. They were severely corroded and in every can considerable areas of the enamel were so completely loosened that they flaked away when the contents of the can were emptied. From the areas on which the enamel had loosened it appeared that the tin was also completely removed. It seemed that the tin was dissolved away or oxidized underneath the enamel, which promptly fell off when the can was emptied. Frequently large areas of the iron appeared exposed.

The principal difference between the exhausted and nonexhausted enameled cans was in the number of swelled cans. The difference in the intensity of the corrosive action was not very marked.

The reenameled cans were much less corroded than the single-enamel cans. However, they had many small areas from which the enamel and the tin had been removed. There were no tightly swelled cans, and only a few flippers in the unexhausted lots. The material in the reenameled cans was of fair quality but distinctly less pleasing than that canned in glass. It had a slightly bitter taste, probably due to some reaction with the enamel or to the presence of iron or tin salts of the organic acids present.

The cans treated by the addition of a sugar sirup instead of water were slightly less corroded, but the differences were not very pronounced. The yellowish coating previously mentioned was sometimes quite apparent in these lots.

The cans packed without the addition of water (treatments 5 and 6) were similar to those from the corresponding lots with water added. The corrosion was more intense but not as great as would have been expected as a consequence of the larger quantity of material in these cans.

The results differed somewhat in the three years. The material purchased in the market at Washington, D. C., seemed to act upon the cans more severely than did the variety grown on the Arlington Experiment Farm.

The question arises as to what constituents are responsible for this severe corrosive action on the can. The results in general point very definitely to the presence of two constituents: (1) Oxalic acid or its salts, accompanied by high total acidity due chiefly to malic acid, and (2) nitrates.

Clough and Clark (8) conclude that corrosion is due to the oxygen content of the porous stems of the rhubarb. Joslyn (17) states that the corrosive action is due to the oxygen content and not to the high acidity. From certain tests made by Kohman (18), he concludes that the substance responsible "is not oxygen but a substance that behaves like oxygen." De Fouw (14) states that nitrates are the only salts that appreciably affect the rate of corrosion of tin by organic acids. Culpepper and Moon (11) have shown that the corrosion of tin cans filled with solutions of organic acids is tremendously increased by the presence of nitrates. Under certain conditions the action occurs without the formation of appreciable amounts of gas. Serger (29) finds that in canned meats nitrates may cause corrosion of the tin container. Culpepper and Moon (13) have shown that oxalic acid is much more corrosive than other organic acids. Although very little free oxalic acid is present in the petioles, it occurs as the acid

potassium salt. Whether the potassium salt would corrode the can in a similar manner is not known. Serger (29) attributes the corrosive action of rhubarb to its oxalic acid content. In the tests here reported the formation of yellowish crystals and a yellowish coating suggests that iron oxalate is one of the products formed. The content of oxalic acid or its salts in rhubarb petioles has been shown by various workers to vary from 0.1 to 0.7 per cent. Culpepper and Moon (12) estimated that there was 6 per cent of air by volume in one sample of rhubarb. The gas content is less than this in the young material and greater than this in the old material. Even in the unexhausted cans this would not be sufficient to account for the amount of corrosion that is apparent.

The analytical results of the present investigation indicate that there is at times as high as 0.1 per cent of nitrate nitrogen in the material. A No. 3 can containing 530 g of rhubarb would contain 0.53 g of nitrate nitrogen, or 3.7 g of potassium nitrate. In cases where there is 0.03 per cent of nitrate nitrogen, which is frequently the case at the stage at which rhubarb is canned, there would be 1.1 g of potassium nitrate. This amount is more than ample to account for the action of the material on the can, especially an acid material like rhubarb. Culpepper and Moon (13) found that oxalic acid in a concentration of 0.5 per cent caused swelling in one lot of plain tin cans. Therefore it would be expected that if oxalic acid alone were responsible the cans would become tightly swelled. In the tests of Kohman (18) the gas formed was not sufficient to swell the can. In the present tests the cans were seldom swelled. The work of Clough and Clark (8) and Kohman (18) shows that the substance responsible for the swelling is highly soluble in water, which is true of most salts of nitric acid. The behavior in general indicates that nitrate nitrogen, occurring as a constituent in the material, is an important cause of corrosion in canned rhubarb.

The analytical results have shown that there is considerable variation in the nitrate content of rhubarb at different ages and in different seasons. Since soils under different cultural practices may contain various amounts of nitrate nitrogen and since the amount in the soil affects the amount in the plant, one must conclude that the nitrate content of the material used by different workers is subject to wide variation. Inasmuch as the oxalic acid also varies it seems reasonable to suppose that these variations may account for some of the differences in the results of canning tests by different workers.

#### SUMMARY

A study has been made of the chemical changes that occur in the petioles of rhubarb with increasing age and changing seasonal conditions. It has been shown that these changes influence the cooking and canning quality of the material.

It was found that the total solids did not vary greatly with age. They were high in the very young petioles, decreased for several days when the petioles were elongating most rapidly, increased slightly during the period of maximum photosynthetic activity, and finally decreased somewhat in the very old material. Marked differences were noted in the samples taken at different seasons. The series

taken in July and October were higher in solids than the series taken in April, May, and June.

The titratable acidity did not vary greatly with age until the petioles were very old, when a marked decrease was noted. There was a very marked difference in the acidity of samples taken at different periods of the season. Acidity was high in the series taken in July and October and low in the series taken in April, May, and June.

Rhubarb is very low in sugars and acid-hydrolyzable polysaccharides and variations in the amounts of these constituents are not particularly significant.

The total nitrogen was highest in the young material and decreased somewhat with age. The amino nitrogen likewise decreased with age. The nitrate nitrogen increased greatly as the age of the material increased. These variations were influenced considerably by seasonal conditions.

Qualitative tests indicate that there are present in the rhubarb petiole pectinlike substances that have considerable influence upon the cooking quality of the material. These substances seem to be largely protopectin.

The low carbohydrate content, the high acid content, and the high moisture content are responsible for many of the characteristics of the material as a food product and explain much of its behavior in cooking.

Rhubarb when packed in plain tin cans had a very severe corrosive action upon the metals of the container. The presence of the resulting metallic compounds had a very pronounced detrimental effect upon the taste and appearance of the material. A high-grade reenameled tin can greatly lessened the amount of corrosion and caused a corresponding improvement in the flavor of the material.

Probably one very important factor in the corrosion is the presence of nitrates. Oxalic acid, accompanied by a high malic acid content, is also thought to be of much importance. The variation due to the age of the material and the seasonal conditions in the amount of nitrates present suggests that differences in the nitrate content may be the cause of the differences in the results obtained by different workers.

The results of the present investigation indicate that rhubarb may be used either for cooking or canning at any time during the growing season if care is taken to select petioles at the proper stage of maturity.

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# PROGRESSIVE CHANGES IN THE CUTICLE OF APPLES DURING GROWTH AND STORAGE<sup>1</sup>

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## INTRODUCTION

In a previous paper (10)<sup>3</sup> on the surface covering of apples the work was confined largely to a study of the progressive changes in the waxlike constituents of apples grown on the Arlington Experiment Farm, Rosslyn, Va. It seemed desirable to extend the study to varieties of apples grown in two dissimilar apple regions, namely, the Wenatchee Valley, Wash., and the Finger Lake district of New York, and to include also quantitative cutin determinations. The results of this work are embodied in the present paper.

## EXPERIMENTAL MATERIAL

The fruit used in this investigation was of the same lot as that described elsewhere (11). Samples of fruit were collected about 55 days after the trees had bloomed and also about the time of picking maturity. A portion of the fruit collected about the time of picking maturity was placed in storage for subsequent work. These samples are referred to, respectively, as early, mature, and storage. Fruit representing the early and the mature stages was sampled at laboratories in the vicinity of the orchards from which it was collected. Material representing the mature and the storage stages of Washington State fruit was picked about a week or 10 days prior to commercial harvesting. The fruit to be stored was packed in boxes with untreated wrappers, shipped under initial ice from Peshastin, Wash., to Philadelphia, and then by express to Washington, D. C. The corresponding samples of New York fruit were picked at commercial harvesting time, packed in lined bushel baskets, and shipped by express to Washington, D. C. Both lots of fruit were placed in the same cold-storage room and kept at 32° F. until removed for analysis.

## METHODS OF ANALYSIS

### PREPARATION OF SAMPLES AND DETERMINATION OF ETHER-SOLUBLE CONSTITUENTS

The method of sampling and preparation was the same as that described previously (10), which briefly is as follows: From the shady side of 75 apples, disks were removed with the aid of a standardized cork borer and immersed for 24 hours in dilute hydrochloric acid. The cuticle was separated from most of the flesh by means of tweezers,

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<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 412.

washed with distilled water, and dried over sulphuric acid. The total ether extract, ursolic acid, and oily fraction were determined according to the methods which have been described in detail. In the previous paper it was stated that the oily fraction consisted mainly of the hydrocarbon triacontane together with the alcohol heptacosanol and small quantities of plastid pigments and other impurities. It has since been shown by Chibnall and his coworkers (1) as well as by the writers (9) that the hydrocarbon is *n*-nonacosane and the alcohol 10-nonacosanol.

#### DETERMINATION OF CUTIN

After the removal of the ether and alcohol soluble constituents from the crude cuticle disks, the residue consisted primarily of cutin<sup>4</sup> admixed with relatively small amounts of impurities derived from the cellular walls and cell contents of adhering epidermal and subepidermal tissues. The presence of such impurities is evidenced from the fact that Rome Beauty cuticle extracted with ether and alcohol and air-dried gave on analysis the following results: Nitrogen, 0.63 per cent (equivalent to 3.94 per cent crude protein); pentosans (Kröber's method), 5.80 per cent; and cellulose, 3.87 per cent. Pectin was present but not quantitatively determined. The presence of lignin could not be shown by the microchemical phloroglucinol-hydrochloric acid test. The quantitative determination of cutin in the presence of these impurities has, in the experience of the writers, been found practically impossible. Even the removal of the impurities in order to determine the cutin residually presented a difficult problem.

König and his coworkers (4, 5), in a study of the chemical components of cellular membranes, particularly the crude-fiber fraction, removed the soluble impurities by treatment of the plant tissues with 2 per cent sulphuric acid in glycerin. After this treatment the residual crude fiber, which consisted of lignin, cellulose, and cutin, was further treated with ammoniacal hydrogen peroxide to oxidize the lignin, and with cuprammonium solution to remove the cellulose. The residue was considered to be cutin. Another method used by König (6) consisted in the removal of the cellulose by treatment with 72 per cent sulphuric acid, which was supposed to leave the lignin and cutin unattacked. The lignin in the insoluble portion was oxidized with ammoniacal hydrogen peroxide and the residue weighed as cutin.

As the above-mentioned methods were not considered adaptable in their entirety to the material at hand, a considerable number of preliminary experiments were made in an attempt to improve them. It was found that treatment with hot dilute acid and alkali, although not drastic enough to affect the cutin, materially reduced the non-cellulosic impurities. For example, Rome Beauty cuticle, previously

<sup>4</sup> A number of investigators have shown that the apple has a firm, thick cuticle which is structureless and covers the epidermis as a continuous layer except for lenticular openings. If the cuticle is separated from adjacent tissues and then extracted with ether, it may be separated into two main portions, the ether-soluble fraction or waxlike coating consisting of a mixture of normal paraffin hydrocarbons, primary and secondary alcohols, and ursolic acid, and the ether-insoluble portion, which is recognized by many and often referred to as cutin. Knowledge of the chemical nature of the cutin of apples is rather meager, although certain analogies in composition may be drawn from the investigations of Freney (2), Freney and Urbain (3), Van Wisselingh (13), Sutthoff (12), Lee (7), and Legg and Wheeler (8), who made studies on cutin from other sources. As a result of this work it may be concluded that cutin consists of an esterlike substance which on saponification yields a number of incompletely identified solid and semiliquid acids.

extracted with ether and alcohol, lost 16.34 per cent impurities when refluxed 30 minutes with 0.2 per cent aqueous potash, followed by 30 minutes, refluxing with 1.25 per cent sulphuric acid. Comparable samples, treated in the same manner, except for allowing the samples to stand overnight in contact with cold aqueous alkali, lost 13.90 per cent in weight. In the latter case, however, the crude cellulose content was slightly higher, which indicated that a portion of the unattacked impurities remained with the cellulose. That nearly all the impurities were removed in the hot alkali and acid treatments was shown by the fact that the oven-dried cutin-cellulose residue contained but 0.1 per cent ash, 0.45 per cent nitrogen, and 1.53 per cent pentosans. Moreover, some of these impurities were associated with the cellulose, as was subsequently determined.

After removal of most of the impurities by hot alkali and acid digestion, treatment of the cutin-cellulose portion with 72 per cent sulphuric acid to remove the cellulose gave a slimy, partly carbonized residue amounting to 47.54 per cent. According to König this residue should represent cutin and lignin, although it is apparent that the percentage is much too low because of the partial decomposition of the cutin by sulphuric acid. Similar cutin-cellulose samples treated with cuprammonium reagent to remove the cellulose gave cutin residues equivalent to 95.75 and 96.23 per cent. In view of the wide divergence in the results of the two methods, neither could be accepted. From the standpoint of accuracy, the cuprammonium method is undoubtedly to be preferred, but it is tedious and lengthy in operation.

It has been known for a long time (2) that cutin is saponified completely with alcoholic potash, and it was thought that the cutin might be quantitatively separated from the cellulose by this means. It was found that saponification for three hours with 3 per cent alcoholic potash gave results quite comparable with the cuprammonium method. For example, triplicate determinations on samples of Rome Beauty cutin-cellulose residues comparable with those used previously gave 95.3, 95.6, and 95.2 per cent cutin by difference. The crude cellulose residue obtained after saponification of the cutin with 3 per cent alcoholic potash gave on analysis 0.1 per cent ash, 0.9 per cent nitrogen (equivalent to 5.62 per cent protein), and 5.16 per cent pentosans. Incidentally the results of the above experiments seem to indicate that the cellulose exists chemically uncombined with the cutin rather than as a definite chemical entity, termed cutocellulose by earlier workers.

Based on preliminary work, part of which is described above, the following empirical method for the determination of the cutin present in apple cuticle was devised and applied throughout the series of experiments herein reported. After extraction of the cuticular disks with ether and alcohol each sample was transferred to a 500-ml Erlenmeyer flask fitted with a ground-glass-joint condenser, refluxed for 30 minutes with 100 ml of 0.2 per cent aqueous potash, filtered through a 35-ml No. 3 Jena-glass crucible with fritted bottom, thoroughly washed with boiling water, and finally dried enough to permit easy removal of the residue from the crucible. The residue was returned to the original flask, refluxed for 30 minutes with 1.25 per cent sulphuric acid, filtered through the same crucible as before, and thoroughly washed with hot water and alcohol. The alkali and

acid treated material was then dried at 110° C. and cooled in a desiccator over sulphuric acid. The weighed cutin-cellulose residue was removed from the crucible and saponified by refluxing for three hours with 100 ml of 3 per cent alcoholic potash. The insoluble cellulosic material was separated by filtration from the hot liquid and washed with hot alcohol and water, after which it was dried to constant weight at 110° C. It was then cooled and weighed. The difference in the two weighings represents the weight of cutin saponified.

TABLE 1.—*Progressive changes in the ether-soluble surface constituents and cutin from the shady side of apples from Washington during maturation and in storage*

Variety and stage of growth	Date of sampling	Age from blooming	Minimum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 mm <sup>2</sup> of surface area				Oily or petroleum ether soluble fraction in total ether extract
					Ursolic acid	Oily or petroleum ether soluble fraction	Total ether extract	Cutin	
FAIL VARIETIES									
McIntosh:		<i>Days</i>	<i>Mm</i>	<i>Mm</i> <sup>2</sup>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Per cent</i>
Early.....	June 28, 1929	55	36-48	29, 218	398.7	137.9	536.6	663.5	25.7
Mature.....	Sept. 4, 1929	123	64-77	27, 901	403.8	206.4	670.2	803.2	30.8
Storage.....	Jan. 3, 1930	244	66-76	27, 911	515.6	289.1	804.7	950.0	35.9
Wagner:									
Early.....	June 30, 1929	57	43-52	28, 887	379.1	199.7	578.8	822.6	34.5
Mature.....	Sept. 17, 1929	136	70-86	27, 780	382.3	294.5	676.8	924.0	43.5
Storage.....	Jan. 3, 1930	244	69-88	27, 762	422.1	300.8	722.9	1, 085.6	41.6
Delicious:									
Early.....	June 27, 1929	54	35-44	28, 664	497.2	217.0	714.2	931.2	30.4
Mature.....	Sept. 25, 1929	144	63-81	27, 902	549.8	296.4	846.2	1, 217.5	35.0
Storage.....	Mar. 5, 1930	305	66-81	27, 884	612.1	529.7	1, 141.8	1, 257.2	46.4
Grimes Golden:									
Early.....	June 28, 1929	55	33-41	29, 503	394.8	213.2	608.0	907.9	35.1
Mature.....	Sept. 4, 1929	123	63-77	27, 898	445.8	386.7	832.5	1, 447.2	46.5
Storage.....	Jan. 17, 1930	258	63-79	27, 541	576.6	513.8	1, 090.4	1, 632.8	47.1
LATE VARIETIES									
Rome Beauty:									
Early.....	June 26, 1929	53	33-42	30, 077	377.1	162.9	540.0	687.6	30.2
Mature.....	Oct. 3, 1929	152	68-88	27, 777	536.4	328.0	864.4	1, 107.9	37.9
Storage.....	Feb. 12, 1930	254	69-89	27, 781	623.5	381.6	1, 005.1	1, 206.4	38.0
Ben Davis:									
Early.....	June 28, 1929	55	35-45	29, 750	440.9	196.3	637.2	841.2	30.8
Mature.....	Sept. 27, 1929	146	63-84	27, 846	494.5	302.3	796.8	858.2	37.9
Storage.....	Feb. 18, 1930	290	67-82	27, 842	623.6	367.4	991.0	938.8	37.1
Arkansas Black:									
Early.....	June 29, 1929	56	35-44	29, 429	500.9	192.6	693.5	972.8	27.8
Mature.....	Oct. 8, 1929	157	67-77	27, 876	631.5	449.8	1, 131.3	1, 295.3	39.8
Storage.....	Mar. 5, 1930	305	70-85	27, 796	836.2	554.8	1, 391.0	1, 346.6	39.9
York Imperial:									
Early.....	June 29, 1929	56	38-42	29, 685	363.9	189.3	553.2	892.1	34.2
Mature.....	Oct. 4, 1929	153	70-80	27, 824	547.0	370.2	917.2	1, 181.7	40.4
Storage.....	Feb. 25, 1930	297	67-77	27, 854	548.2	412.8	961.0	1, 185.2	43.0
Stayman Winesap:									
Early.....	June 26, 1929	53	35-42	29, 776	497.7	193.4	691.1	1, 041.6	28.0
Mature.....	Sept. 28, 1929	152	66-87	27, 813	535.6	381.1	916.7	1, 416.1	41.6
Storage.....	Mar. 13, 1930	313	69-81	27, 805	601.2	446.3	1, 047.5	1, 481.8	42.6
Winesap:									
Early.....	June 27, 1929	54	33-38	30, 342	491.1	175.3	666.4	923.9	26.3
Mature.....	Oct. 7, 1929	156	63-75	27, 976	531.1	391.7	922.8	1, 473.2	42.4
Storage.....	Mar. 20, 1930	320	64-73	27, 970	599.2	490.5	1, 089.7	1, 467.9	45.0
Northern Spy:									
Mature.....	Sept. 5, 1929	124	63-80	28, 254	349.6	278.2	627.8	972.5	44.3
Storage.....	Feb. 12, 1930	284	67-81	27, 854	409.3	348.2	757.5	1, 119.5	46.0
Esopus Spitzenburg:									
Early.....	June 27, 1929	54	36-43	29, 629	415.1	176.9	592.0	898.1	29.9
Mature.....	Sept. 26, 1929	145	66-78	27, 861	595.1	479.8	1, 074.9	1, 415.5	44.6
Storage.....	Jan. 17, 1930	258	67-77	27, 877	666.5	505.7	1, 172.2	1, 370.8	43.1
Yellow Newtown:									
Early.....	June 30, 1929	57	38-47	29, 350	367.3	194.9	562.2	920.2	34.7
Mature.....	Oct. 4, 1929	153	66-84	27, 845	401.1	379.6	780.7	1, 047.1	48.6
Storage.....	Apr. 17, 1930	348	66-83	27, 854	456.7	452.3	909.0	1, 055.8	49.8

\* Mixed sample; no difference discernible between shady and sunny sides of same fruit.

TABLE 2.—*Progressive changes in the ether-soluble surface constituents and cutin from the shady side of apples from New York during maturation and in storage*

Variety and stage of growth	Date of sam- pling	Age from bloom- ing	Mini- mum and maxi- mum diam- eters of ap- ples used	Total area of 75 disks	Constituents per 100,000 mm <sup>2</sup> of surface area				Oily or petro- leum ether soluble fraction in total ether extract
					Ursolic acid	Oily or petro- leum ether soluble fraction	Total ether extract	Cutin	
FALL VARIETIES									
McIntosh:		<i>Days</i>	<i>Mm</i>	<i>Mm</i> <sup>2</sup>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Per cent</i>
Early.....	July 6, 1929	52	31-41	29,386	337.9	143.3	481.2	596.5	29.8
Mature.....	Sept. 24, 1929	132	56-68	28,129	493.1	251.7	744.8	873.1	33.8
Storage.....	Jan. 3, 1930	233	57-68	28,129	578.8	289.7	868.5	980.3	33.4
Wagener:									
Early.....	July 6, 1929	52	31-38	29,993	361.4	168.4	529.8	679.8	31.8
Mature.....	Oct. 10, 1929	148	53-70	28,227	503.1	353.6	856.7	799.3	41.3
Storage.....	Jan. 3, 1930	233	58-70	28,100	565.5	381.2	946.7	1,076.8	40.3
Delicious:									
Early.....	July 8, 1929	52	30-38	30,820	410.7	226.7	637.4	738.6	35.6
Mature.....	Oct. 14, 1929	150	49-61	28,400	617.6	491.2	1,108.8	1,011.9	44.3
Storage.....	Mar. 5, 1930	292	51-67	28,223	637.4	517.3	1,154.7	1,121.8	44.8
Grimes Golden:									
Early.....	July 10, 1929	55	26-36	32,286	397.0	232.6	629.6	871.2	36.9
Mature.....	Sept. 30, 1929	137	50-59	28,472	466.7	455.9	922.6	1,426.6	49.4
Storage.....	Dec. 17, 1929	215	50-61	28,450	599.3	477.0	1,076.3	1,663.0	44.2
LATE VARIETIES									
Rome Beauty:									
Early.....	July 12, 1929	53	30-40	30,667	375.0	191.1	566.1	733.1	33.8
Mature.....	Oct. 18, 1929	151	51-67	28,261	531.8	353.1	884.9	1,091.1	39.9
Storage.....	Feb. 12, 1930	268	62-76	27,941	579.8	407.6	987.4	-----	41.3
Ben Davis:									
Early.....	July 10, 1929	54	28-40	31,016	402.7	215.7	618.4	804.1	34.9
Mature.....	Oct. 17, 1929	153	53-67	28,210	759.7	404.1	1,163.8	1,166.0	34.7
Storage.....	Feb. 20, 1930	279	57-70	28,098	814.6	544.2	1,358.8	1,463.5	40.1
Arkansas:									
Early.....	July 16, 1929	58	39-49	29,180	510.3	208.3	718.6	1,013.7	29.0
Mature.....	Oct. 8, 1929	142	65-85	27,866	573.2	303.6	876.8	1,254.0	34.6
Storage.....	Jan. 21, 1930	247	63-87	27,862	672.9	365.0	1,037.9	1,492.4	35.2
Baldwin:									
Early.....	July 8, 1929	53	34-44	29,609	410.6	189.1	599.7	864.2	31.5
Mature.....	Oct. 14, 1929	151	57-75	28,075	560.6	354.8	915.4	1,072.2	38.8
Storage.....	Feb. 18, 1930	278	58-76	28,010	646.9	489.8	1,136.7	-----	43.1
Stayman Winesap:									
Early.....	July 16, 1929	59	33-42	30,203	526.4	226.2	752.6	1,036.7	30.1
Mature.....	Oct. 8, 1929	143	51-72	28,148	647.4	365.6	1,013.0	1,386.4	36.1
Storage.....	Mar. 13, 1930	299	55-73	28,137	801.4	456.3	1,257.7	-----	36.3
Winesap:									
Early.....	July 16, 1929	57	29-38	31,408	495.4	228.3	723.7	965.1	31.5
Mature.....	Oct. 8, 1929	141	54-62	28,281	610.3	329.9	940.2	1,247.5	35.1
Storage.....	Mar. 20, 1930	304	50-62	28,340	713.7	449.8	1,163.5	1,589.2	38.7
Northern Spy:									
Early.....	July 12, 1929	52	32-50	29,704	353.8	190.2	544.0	637.5	35.0
Mature.....	Oct. 18, 1929	150	53-76	28,138	500.4	340.5	840.9	957.8	40.5
Storage.....	Feb. 12, 1930	267	54-76	27,723	560.5	373.7	934.2	1,131.2	40.0
Esopus Spitzenburg:									
Early.....	July 9, 1929	53	29-37	31,097	417.8	218.0	635.8	888.2	34.3
Mature.....	Sept. 30, 1929	136	53-67	28,243	558.4	451.8	1,010.2	1,257.8	44.7
Storage.....	Dec. 17, 1929	214	50-64	28,325	685.2	462.8	1,148.0	1,407.4	40.3
Yellow Newtown:									
Early.....	July 12, 1929	53	29-38	31,101	345.9	216.7	562.6	870.0	38.5
Mature.....	Oct. 17, 1929	150	47-57	28,591	508.3	354.7	863.0	893.7	41.1
Storage.....	Mar. 5, 1930	289	48-56	28,551	491.7	389.4	881.1	1,007.4	44.2
Rhode Island Green- ing:									
Early.....	July 9, 1929	53	34-41	29,184	354.2	187.4	541.6	739.3	34.6
Mature.....	Sept. 25, 1929	131	56-70	28,067	476.4	327.1	803.5	1,104.5	40.7
Storage.....	Jan. 21, 1930	249	59-72	28,043	557.7	369.4	927.1	1,308.6	39.8

## PROGRESSIVE CHANGES IN WAXLIKE CONSTITUENTS AND CUTIN DURING GROWTH AND STORAGE

Data reported in Tables 1 and 2 represent the early, mature, and storage stages for the shady side of fruit grown in Washington State and in New York. In the previous paper (10) it was shown that the quantities of ursolic acid, oily fraction, and total ether extract present

at maturity and at the end of the storage period were invariably greater than those found in the early stage of growth, and that in general the constituents increased from the time of picking to the end of the storage period. The results reported in the present paper confirm the previous conclusions. It was also shown that while the quantities of both ursolic acid and the oily fraction became larger with increasing maturity, the oily fraction increased at a faster rate than did the ursolic acid; hence with advancing maturity there was in general a progressive increase in the percentage of the oily fraction in the total ether extract. This condition with one exception (Ben Davis from New York) is also found to be true in the present study.

In connection with the cutin determinations reported in the same tables it is found that the same general conclusions can be drawn as in the case of the waxlike constituents. For example, the quantities of cutin were found to be greater at maturity and at the end of the storage period than in the early stage. With two exceptions (Winesap and Esopus Spitzenburg from Washington), the quantities of cutin found at the end of the storage period were greater than at picking maturity. It is not clear why in many cases there should be unusually large differences in the quantities of cutin found at the end of the storage period as compared with those found at picking maturity, since it is difficult to conceive how cutin would continue to be formed after the fruit has been picked. It is easy to understand how the oily fraction and the ursolic acid might increase during storage, since it is entirely possible that the constituents representing the oily fraction may diffuse outward from the interior of the fruit and that ursolic acid may be formed as a result of oxidation or condensation of cell-wall progenitors. Of course the latter hypothesis may also afford an explanation of the observed cutin increases during the storage period.

#### COMPARISON BETWEEN FRUIT GROWN IN WASHINGTON AND NEW YORK

At the mature stage the mean values, in milligrams per 100,000 mm<sup>2</sup> of surface area, for only those varieties grown in both Washington and New York, are, respectively: Ursolic acid, 480.5 and 563.3; oily fraction, 338.6 and 377.5; total ether extract, 819.1 and 940.8; cutin, 1,152.9 and 1,095.6; percentage of oily fraction in total ether extract, 41.3 and 40.1. At the storage stage the corresponding values are: Ursolic acid, 555.1 and 638.9; oily fraction, 420.5 and 431.7; total ether extract, 975.6 and 1,070.6; cutin, 1,233.3 and 1,271.2; percentage of oily fraction in total ether extract, 43 and 40.3. These figures indicate that the New York apples, when considered as a group, had higher amounts of ursolic acid, oily fraction, and total ether extract at the mature stage and at the end of the storage period than did the Washington apples. On the other hand, the percentage of oily fraction in the total ether extract was higher for both stages in the Washington fruit.

When individual varieties are considered it is found that, with the exception of Esopus Spitzenburg and Rome Beauty, ursolic acid is higher for the New York than for the corresponding Washington fruit. Likewise all New York varieties are higher in oily fraction and total ether extract except Esopus Spitzenburg, Winesap, Stayman Winesap, and Yellow Newtown, which are lower in oily fraction, and Esopus Spitzenburg, which is lower in total ether extract.



It should be borne in mind that variations in total quantities of constituents may be due to (1) differences in the average daily rate of deposition for the same length of growing season, or to (2) differences in length of growing period where the average daily rate of deposition is the same. For example, the average daily rates of deposition, which are given in column 6, Table 3, for the total ether extract representing Yellow Newtown from Washington and New York in 1929 for practically the same period of growth (153 and 150 days), are 5.10 and 5.75 mg, respectively, thereby resulting in a lower total ether extract for Washington fruit than for New York fruit (780.7 and 863 mg). On the other hand, in the case of Grimes Golden from the two localities the average daily rates of deposition are practically the same (6.76 and 6.73 mg.), whereas the lengths of growing season are 123 and 137 days and the quantities of total ether extract 832.5 and 922.6 mg for Washington and New York, respectively.

TABLE 3.—Comparative data representing the mature stage, shady side, for fall and late varieties of apples from several localities

Variety, locality, and year	Age from blooming	Constituents per 100,000 mm <sup>2</sup> of surface area					Oily or petroleum ether soluble fraction in total ether extract
		Ursolic acid	Oily or petroleum ether soluble fraction	Ether extract		Cutin	
				Total	Average daily rate of deposition		
FALL VARIETIES							
McIntosh:	<i>Days</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Per cent</i>
Virginia, 1927.....	149	596.8	284.1	880.9	5.91	-----	32.3
British Columbia, 1927.....	-----	807.6	420.3	1,227.9	-----	-----	34.2
Washington, 1929.....	123	463.8	206.4	670.2	5.45	803.2	30.8
New York, 1929.....	132	493.1	251.7	744.8	5.64	873.1	33.8
Jonathan:							
Virginia, 1926.....	149	567.3	324.1	891.4	5.98	-----	36.4
Washington, 1927.....	-----	542.8	299.6	842.4	-----	-----	35.6
Washington, 1929 <sup>a</sup> .....	151	555.1	352.0	907.1	6.01	1,094.7	38.8
Do. <sup>b</sup> .....	150	511.1	274.9	786.0	5.24	853.7	35.0
Do. <sup>c</sup> .....	153	481.0	321.5	802.5	5.24	946.4	40.0
Do. <sup>d</sup> .....	153	538.1	351.2	889.3	5.81	929.0	39.5
Winter Banana:							
Virginia, 1926.....	132	525.2	312.9	838.1	6.35	-----	37.3
Virginia, 1927.....	141	487.5	308.0	795.5	5.64	-----	38.7
Washington, 1927.....	-----	619.1	443.5	1,062.6	-----	-----	41.7
Delicious:							
Virginia, 1926.....	160	505.7	398.8	904.5	5.65	-----	44.1
Virginia, 1927.....	154	747.6	448.7	1,196.3	7.76	-----	37.5
Washington, 1927.....	-----	437.5	269.1	706.6	-----	-----	38.1
Washington, 1929.....	144	549.8	296.4	846.2	5.88	1,217.5	35.0
New York, 1929.....	150	617.6	491.2	1,108.8	7.39	1,011.9	44.3
Wagener:							
Washington, 1929.....	136	382.3	294.5	676.8	4.98	924.0	43.5
New York, 1929.....	148	503.1	353.6	856.7	5.79	799.3	41.3
Grimes Golden:							
Virginia, 1926.....	167	572.6	610.1	1,182.7	7.08	-----	51.6
Virginia, 1927.....	152	588.9	500.6	1,089.5	7.16	-----	45.9
Washington, 1927.....	-----	658.2	421.0	1,079.2	-----	-----	39.0
Washington, 1929.....	123	445.8	386.7	832.5	6.76	1,447.2	46.5
New York, 1919.....	137	466.7	455.9	922.6	6.73	1,426.6	49.4

<sup>a</sup> Yakima, sprayed with lead arsenate and oil.

<sup>b</sup> Yakima, unsprayed.

<sup>c</sup> Wenatchee, unsprayed.

<sup>d</sup> Wenatchee, sprayed with lead arsenate.

TABLE 3.—Comparative data representing the mature stage, shady side, for fall and late varieties of apples from several localities—Continued

Variety, locality, and year	Age from blooming	Constituents per 100,000 mm <sup>2</sup> of surface area					Oily or petroleum ether soluble fraction in total ether extract
		Ursolic acid	Oily or petroleum ether soluble fraction	Ether extract		Cutin	
				Total	Average daily rate of deposition		
LATE VARIETIES							
Baldwin:	<i>Days</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Per cent</i>
Virginia, 1927.....	153	579.4	322.6	902.0	5.90	-----	35.8
New York, 1929.....	151	500.6	354.8	915.4	6.06	1,072.2	38.8
Stayman Winesap:							
Washington, 1929.....	152	535.6	381.1	916.7	6.03	1,416.1	41.6
New York, 1929.....	143	647.4	365.6	1,013.0	7.08	1,386.4	36.1
Arkansas Black:							
Virginia, 1926.....	149	722.3	395.6	1,117.9	7.50	-----	35.4
Virginia, 1927.....	159	624.5	397.2	1,021.7	6.43	-----	38.9
Washington, 1927.....	-----	542.5	392.3	934.8	-----	-----	42.0
Washington, 1929.....	157	681.5	449.8	1,131.3	7.20	1,295.3	39.8
Ben Davis:							
Virginia, 1926.....	180	502.7	400.2	902.9	5.01	-----	44.3
Washington, 1929.....	146	494.5	302.3	796.8	5.46	858.2	37.9
New York, 1929.....	153	759.7	404.1	1,163.8	7.61	1,106.0	34.7
Rome Beauty:							
Virginia, 1926.....	183	552.8	382.2	935.0	5.11	-----	40.9
Washington, 1929.....	152	536.4	328.0	864.4	5.69	1,107.9	37.9
New York, 1929.....	151	531.8	353.1	884.9	5.86	1,091.1	39.9
Winesap:							
Washington, 1929.....	150	640.8	360.3	1,001.1	6.67	1,678.2	36.0
Do. *.....	150	542.5	455.3	997.8	6.65	1,506.2	45.6
Do. *.....	156	531.1	391.7	922.8	5.91	1,473.2	42.4
New York, 1929.....	141	610.3	329.9	940.2	6.67	1,247.5	35.1
Northern Spy:							
Washington, 1929.....	124	349.6	278.2	627.8	5.06	972.5	44.3
New York, 1929.....	150	500.4	340.5	840.9	5.61	957.8	40.5
Rhode Island Greening:							
Virginia, 1927.....	152	463.0	366.5	829.5	5.46	-----	44.2
New York, 1929.....	131	476.4	327.1	803.5	6.13	1,104.5	40.7
Yellow Newtown:							
Washington, 1929.....	158	611.5	527.7	1,139.2	7.21	1,327.1	46.3
Do. *.....	158	534.9	400.7	935.6	5.92	1,005.8	42.8
Do. *.....	153	401.1	379.6	780.7	5.10	1,047.1	48.6
New York, 1929.....	150	508.3	354.7	863.0	5.75	893.7	41.1
York Imperial:							
Virginia, 1926.....	187	514.2	470.0	984.2	5.26	-----	47.8
Virginia, 1927.....	180	558.4	506.4	1,064.8	5.91	-----	47.6
Washington, 1929.....	153	547.0	370.2	917.2	5.99	1,181.7	40.4
Esopus Spitzenburg:							
Washington, 1927.....	-----	474.0	451.5	925.5	-----	-----	48.8
Washington, 1929.....	145	595.1	479.8	1,074.9	7.41	1,415.5	44.6
New York, 1929.....	136	558.4	451.8	1,010.2	7.43	1,257.8	44.7

\* Yakima, unsprayed.

† Yakima, sprayed with lead arsenate and oil.

‡ Different strain from normal variety.

§ Different strain from ‡ and normal variety.

¶ Normal variety.

It should also be noted that the average daily rate of deposition of the total ether extract for individual varieties is higher for the same varieties grown in New York during 1929 than for those grown in Washington, except for Grimes Golden, in which case the value is practically the same for both localities.

#### COMPARATIVE DATA REPRESENTING THE MATURE STAGE FOR EACH VARIETY FROM SEVERAL LOCALITIES

In Table 3 a summary is given of data, including some previously reported results, representing quantities of constituents and percentage of oily fraction in the total ether extract found on the shady side of the fruit at picking maturity for all varieties that were collected

from two or more localities. These data make it possible to observe more clearly than hitherto the variations for individual varieties that occur as a result of somewhat different environmental conditions and, to a lesser extent, differences due to variations in length of growing period. The localities and seasons of growth represented by the various samples include the irrigated Wenatchee and Yakima Valleys, an unusually dry summer in the Finger Lake district, and a normal and dry summer at the Arlington farm in Virginia.

Since the environments may be considered as somewhat extreme, the range in values for the ether-soluble constituents for a particular variety in most cases may be considered to include the most probable values for these substances. The table shows that some varieties like Rome Beauty, Baldwin, and Rhode Island Greening show only small variations in the values of the ether-soluble constituents despite considerable variations in growing conditions, while others like Grimes Golden, Delicious, and McIntosh show considerable variations in values.

In column 6, of Table 3, the average daily rates of deposition of total ether extract are given for those varieties for which complete data are available. These values reflect variations due to environment independent of the length of the period of growth and may, therefore, be more characteristic of varieties than is the total quantity of the ether extract.

When the total ether extract and cutin are averaged for the varieties for which data are available, the ratio of total ether extract to cutin is in the proportion of about 44 to 56. In other words, the main outer protective coating of apples is made up of approximately 44 per cent of ether-soluble materials and 56 per cent of ether-insoluble cutin.

#### SUMMARY AND CONCLUSIONS

Further studies on the progressive changes in the waxlike coating on the surface of the shady side of the apple during growth and storage have been made on varieties grown in two dissimilar apple regions, namely, the Wenatchee Valley, Wash., and the Finger Lake district of New York. A method for the quantitative determination of cutin, present in the cuticle of apples, has been devised and used in the present study.

<sup>2</sup> In confirmation of previous results, obtained from fruit grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., it was found with New York and Washington fruit that quantities of ursolic acid, oily fraction, and total ether extract present at maturity and at the end of the storage period were greater than those found in the early stage of growth and that the quantities of the constituents increased from the time of picking to the end of the storage period. It was also found that with advancing maturity there was in general a progressive increase in the percentage of the oily fraction in the total ether extract.

Quantities of cutin were greater at maturity and at the end of the storage period than in the early stage. The proportion of total ether extract to cutin varies considerably in individual varieties, but when these substances representing the mature stage are averaged for the varieties for which complete data are available, the ratio of total ether extract to cutin is about 44 to 56, indicating that the cuticle of apples, which constitutes the main outer protective coating, is com-

posed of approximately 44 per cent of ether-soluble constituents and 56 per cent of ether-insoluble cutin.

In considering comparable apples from each locality as a group, New York fruit in 1929 had higher amounts of ursolic acid, oily fraction, and total ether extract at the mature stage and at the end of the storage period than did Washington apples. Individually the same varieties from the two localities varied somewhat with respect to quantities of constituents.

Data are given showing the quantities of constituents and percentage of oily fraction in the total ether extract at picking maturity for all varieties collected from two or more localities and the average daily rates of deposition of total ether extract for most of the varieties. These values may possibly be characteristic of varieties, since they reflect differences in quantities of total ether extract due to environment and hereditary tendencies independent of the length of growing season.

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# INHERITANCE OF BUNT REACTION AND OTHER CHARACTERS IN HOPE WHEAT CROSSES <sup>1</sup>

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## INTRODUCTION

Bunt, or stinking smut, caused by *Tilletia tritici* (Bjerk.) Wint. or by *T. levis* (Kuehn), long has been a serious disease of wheat in the Pacific Northwest. In recent years it has become an important problem in Montana. Winter wheat is more severely affected by bunt than is spring wheat, but since the commercial distribution of certain new varieties, particularly Kota, Supreme, and Ceres, bunt has become more prevalent in spring wheat.

A study has been made of bunt reaction and certain other characters in the parents and F<sub>2</sub> hybrid strains of crosses between Hope wheat and three other varieties, namely, Ceres, Marquis, and Hard Federation. The present paper reports the results of this investigation.

## REVIEW OF LITERATURE

Inherent differences in the reaction of wheat varieties to bunt were reported by Farrer (7)<sup>2</sup> in 1901. Since then varietal reaction has been investigated and reviewed by several workers (11, 13, 14). An almost complete range of reaction has been obtained with different varieties, varying from immune and resistant to susceptible, each inherently different but with some overlapping. No immune variety of common spring wheat has been found. In winter-wheat hybrids immunity is inherited as a dominant character in contrast with resistance, which is inherited as a recessive character. Immunity from some forms of bunt, or stinking smut, has been obtained in Martin and Hussar, two winter wheats. In crosses studied by Briggs (2, 3) immunity has been found to segregate in simple Mendelian ratios, the result of one or two genetic-factor differences, respectively. In other studies, where different degrees of resistance and susceptibility are involved, the results have not been so definite and indicate that several factors are involved.

Different physiologic forms of bunt have been reported recently by Gaines (8) and Rodenhiser (12). In some sections new forms have increased the problems of the plant breeder.

## MATERIAL AND METHODS

Marquis, the principal commercial variety of hard red spring wheat grown in Montana, has some resistance to bunt, and partly for this reason the losses in spring wheat have been less serious than those in

<sup>1</sup> Received for publication July 29, 1932; issued April, 1933. The work here reported was done in cooperation with the Montana Agricultural Experiment Station. This paper was read at the meeting of the western section of the American Society of Agronomy at Bozeman, Mont., July 31, 1930.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 425.

winter wheat. Hope spring wheat has a lower percentage of bunt or a greater resistance than Marquis. This stronger resistance of Hope has been studied in crosses involving the weaker resistance of Marquis and different degrees of susceptibility in Ceres and Hard Federation. Three crosses have been tested with a collection of *Tilletia levis* from Bozeman, Mont.

Since the presence of more than one physiologic form of bunt would complicate the problem, a preliminary study was made of the material used in the present investigation. In 1929 collections of bunt from various localities in Montana were tested individually on the parent wheat varieties at Bozeman. Since no differential reactions were obtained, the collections were assumed to represent only one form.

The parent wheat varieties and the  $F_2$  hybrid progenies were grown at the Montana Agricultural Experiment Station, Bozeman, Mont., in 1929. With one exception, the  $F_2$  material was grown, under bunt-free conditions, at the United States Northern Great Plains Field Station, Mandan, N. Dak., in 1928. The  $F_2$  plants of the Hope  $\times$  Hard Federation cross were grown in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.), in the winter of 1928-29.

The seed of the parent varieties and of the  $F_2$  plants was inoculated with *Tilletia levis* by shaking an excess quantity of spores and the seed wheat in a glass container. The  $F_3$  hybrid and parent plants were grown in rod rows 1 foot apart and were spaced 3 inches apart in each row. The parent checks occupied every tenth row, and the  $F_3$  strains, arranged in the random order of the harvesting of the  $F_2$  plants, occupied the intervening rows.

After harvest, the individual plants in each row were studied for bunt infection. The studies on the Hope  $\times$  Marquis and Hope  $\times$  Ceres crosses were made in the field at Bozeman, while those on the Hope  $\times$  Hard Federation cross were made in the laboratory at Washington, D. C. The latter cross was more closely studied than the others, as each apparently bunt-free plant was threshed separately, and if a trace of bunt was observed during or after threshing, the plant was classified as bunted.

## EXPERIMENTAL DATA

### INHERITANCE OF REACTION TO BUNT

The parent reaction in percentage of bunted plants and the segregation in the three crosses, Hope  $\times$  Marquis, Hope  $\times$  Ceres, and Hope  $\times$  Hard Federation, as shown by the average of  $F_3$  hybrid strains, are shown in Table 1, and graphically in Figure 1.

The data on the hybrids show a segregation covering almost the complete range of the parents, except that in the Hope  $\times$  Hard Federation cross there were no  $F_3$  hybrid strains within the limits of the Hope parent. In this cross there is a preponderance of very susceptible strains. The absence of strains having the strong resistance of the Hope parent indicates that in this cross smut reaction is determined by a larger number of genetic factors than in the other crosses. Apparently a sufficient number of strains was not grown to recover the Hope genotype. In the Hope  $\times$  Ceres cross an intermediate inheritance was indicated, 12 of the 103 strains having a

reaction similar to that of the Hope parent. At the other end of the curve there were 18 strains within the limits of the Ceres parent. These results indicate that at least two genetic factors for bunt reaction are involved in this cross, since on the basis of a 1-factor difference, one-fourth, or about 26 strains, would be expected to fall within the range of each parent.

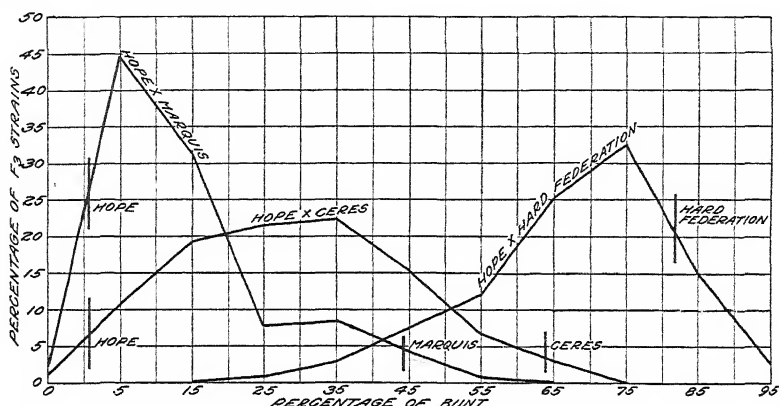


FIGURE 1.—Frequency distribution of the average percentage of bunted plants of  $F_3$  hybrid strains for three wheat crosses, together with the average of the four parents, at Bozeman, Mont., 1929

TABLE 1.—Percentages of bunted wheat plants in rows of Hope, Marquis, Ceres, and Hard Federation parents and  $F_3$  hybrid strains of crosses between Hope and these other varieties grown at Bozeman, Mont., in 1929.

Percentage of bunted plants	Number of rows of indicated parent				Number of rows of $F_3$ hybrid strains of indicated cross		
	Hope	Marquis	Ceres	Hard Federation	Hope x Marquis	Hope x Ceres	Hope x Hard Federation
0.0.....	7				2	1	
2.5.....	9				19	2	
7.5.....	2				22	9	
12.5.....					6	6	
17.5.....					20	14	
22.5.....					4	11	1
27.5.....					3	11	1
32.5.....					5	16	
37.5.....		2			3	7	3
42.5.....		1			2	8	2
47.5.....		2	1		2	8	6
52.5.....		1			1	4	5
57.5.....			2			3	8
62.5.....			1			2	8
67.5.....				1		1	19
72.5.....			1				15
77.5.....				2			20
82.5.....							9
87.5.....			1	2			7
92.5.....				1			3
97.5.....							
Total.....	18	6	6	6	92	103	107
Average %.....	2.1	44.2	64.2	81.7	14.7	29.3	68.5
P. E.....	±.4	±1.7	±3.9	±2.7	±.9	±1.0	±.9

\* Average percentage of bunted plants.

In the Hope x Marquis cross there is a preponderance of strains approaching the stronger resistance of the Hope parent. Of the 92 strains, 43 were within the limits of the Hope parent; only 8 strains

were within the limits of the Marquis parent. It is recognized that the number of parent check rows was comparatively small and may not give a true expression of the parental range.

None of these data indicates a simple single-factor difference, or a 1:2:1 segregation, as found by Briggs (2) in crosses with the immune variety Martin. In general, the results from the three crosses indicate that the stronger the degree of resistance involved in the hybrids the less complicated the inheritance, with an increasing tendency away from an imperfect dominance of susceptibility to an imperfect dominance of strong resistance.

#### SEGREGATION OF VARIOUS CHARACTERS IN HOPE × HARD FEDERATION

In addition to data on percentage of bunted plants, other data were obtained on the Hope × Hard Federation cross, including the percentage of bunt, yield per plant, glume color, kernel color, and awnedness. These data are presented in the order given, together with the relation between some of these characters.

#### PERCENTAGE OF BUNT

The percentage of bunt was determined by separating the total number of heads from each plant into bunt-free, partly bunted, and fully bunted groups. In computing the percentage of bunt per plant, an attempt was made to weight the results according to whether a head was fully or partly bunted. The number of fully bunted heads was multiplied by 1 and the partly bunted heads by 0.5. The sum of these products was then divided by the total number of heads, giving a percentage based on the severity of infection. The average percentage of bunt in each strain or parent-check row was then determined from the total number of plants. The percentage of bunt thus determined is considerably lower than the percentage of bunted plants. The comparable data are shown in Table 2.

TABLE 2.—Percentage of bunt compared with percentage of infected plants in rows of the Hope and Hard Federation parents and 107  $F_2$  hybrid strains grown at Bozeman, Mont., in 1929

Percentage of bunt	Number of rows of—			Percentage of bunted plants	Number of rows of—		
	Hope	$F_2$ strain	Hard Federation		Hope	$F_2$ strain	Hard Federation
2.5	5	6		2.5	3		
7.5		18		7.5	2		
12.5		17		12.5			
17.5		25		17.5			
22.5		21		22.5		1	
27.5		11		27.5		1	
32.5		6		32.5			
37.5		2	1	37.5		3	
42.5		1	1	42.5		2	
47.5			2	47.5		6	
52.5			2	52.5		5	
57.5				57.5		8	
62.5				62.5		8	
67.5				67.5		19	1
72.5				72.5		10	
77.5				77.5		25	2
82.5				82.5		9	
87.5				87.5		7	2
92.5				92.5		3	1
Total	5	107	6	Total	5	107	6
Average	2.5	17.6	46.7	Average	4.5	68.5	81.7
P. E.	0	±.6	±1.6	P. E.	±.8	±.9	±2.5

\* Average percentage of bunt or bunted plants.



The data in Table 2 show that in the hybrids the average percentage of bunt was only about one-fourth as great as the percentage of infected plants. The percentage of bunt measures the effect of bunt infection on yield, whereas the percentage of infected plants is used to determine the inheritance of bunt reaction. The correlation between the percentage of bunt and the percentage of infected plants is positive,  $r = +0.741 \pm 0.029$ . This is both important and significant from a plant-breeding standpoint, indicating that the two measures are closely related.

#### YIELD OF GRAIN

The heads from each plant were threshed and the yield of grain per plant was determined in grams. The total of these yields was then divided by the number of plants in each row. There was some variation in the number of plants per row, but it was felt that the variation was not serious enough to influence the results in general. These average yields are presented in frequency classes in Table 3, where the average yields for the parent checks and hybrid strains also are shown. The average yields of the hybrids approach those of the resistant Hope parent. There was a wide and significant difference between the parents, although the average yields of the bunt-free plants of the parent checks did not show so much difference, those of Hope averaging 10.6 g<sup>3</sup> and those of Hard Federation 9.9 g. As shown in Table 3, the average yield of the smut-free hybrid plants was  $11.8 \pm 0.1$  g and that of the bunted hybrid plants  $8.8 \pm 0.1$  g. The difference of  $3.0 \pm 0.1$  g is statistically significant and represents an average loss in yield of 20.5 per cent.

TABLE 3.—Average plant yield of rows of Hope and Hard Federation parents, of 107 F<sub>3</sub> hybrid strains, and of infected and bunt-free hybrid plants grown at Bozeman, Mont., in 1929

Yield (grams)	Number of rows of—				
	Hope	F <sub>3</sub> strains	Hard Federation	Infected hybrid plants	Bunt-free hybrid plants
4.5.....			3	2	
5.5.....		2		4	
6.5.....		3	3	6	
7.5.....		11		26	2
8.5.....		21		21	7
9.5.....	1	25		23	7
10.5.....	3	24		15	19
11.5.....	1	12		7	24
12.5.....		7		1	23
13.5.....		1		2	14
14.5.....		1			6
15.5.....					1
16.5.....					4
Total.....	5	107	6	107	107
Average <sup>a</sup> .....	10.5	9.7	5.5	8.8	11.8
P. E.....	$\pm 1$	$\pm 1$	$\pm 3$	$\pm 1$	$\pm 1$

<sup>a</sup> Average plant yield of all rows.

<sup>3</sup> g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

The relation between the percentage of bunt and the yield per plant is shown in the correlation surface in Table 4. The correlation ( $r = -0.554 \pm 0.045$ ) shows a negative relation between percentage of bunt and yield. This correlation coefficient also indicates that about 30.7 per cent of the squared variability of yield was caused by the variation in percentage of bunt present.

TABLE 4.—Correlation between percentage of bunt and average yield per plant of 107  $F_3$  strains of Hope  $\times$  Hard Federation wheat crosses grown at Bozeman, Mont., in 1929

Percentage of bunt	Number of strains having indicated average yield (grams) per plant										Total
	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	
2.5						1	3	2			6
7.5			1	2	3	8	1	3			18
12.5		1	1	1	6	3	4	1			17
17.5				7	11	2	2	1		1	26
22.5			5	6	2	5	2				20
27.5		1	1	2	6				1		11
32.5	1		2	3							6
37.5	2										2
42.5	1										1
Total	4	2	10	21	28	21	12	7	1	1	107

$$r = -0.554 \pm 0.045.$$

An effort was made to determine the decrease in yield caused by the increase of bunt. There is a strong negative relation between the percentage of bunt and yield. This correlation is not entirely correct, however, since the  $F_3$  strains were not all genetically alike in

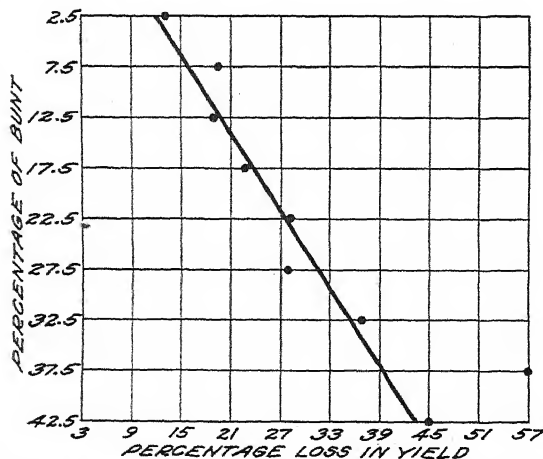


FIGURE 2.—Regression line showing relation between percentage of bunt and loss in yield. Each increase of 5 per cent in bunt caused a decrease of about 4 per cent in yield

yielding ability. Since each hybrid row contained both clean and bunted plants, it was possible to obtain a measure of the yield of each group. It was then possible to determine the percentage of loss in yield caused by the infection of bunt.

The correlation between the percentage of bunt and the percentage loss in yield was found to be

$$r = +0.501 \pm 0.049.$$

Figure 2 shows the regression of percentage loss in yield on the percentage of bunt.

Dots indicate the mean loss for each of the bunt classes. The regression line and the dots coincide very well except for the last two classes. In the 37.5 per cent bunt class there were only two observations and in the 42.5 per cent class only one. These small numbers account

in part for the variation. According to these data an increase of 5 per cent in bunt caused a decrease of about 4 per cent in yield.

#### GLUME COLOR

Glume colors of wheat are most commonly classed as white and brown. Biffen (1) first reported brown glume color dominant to white in the single 3:1 or 1:2:1 ratio. Although there often is considerable variation in the intensity of the brown color, no other ratio has been reported for common wheats.

Glume color was not described in the  $F_2$  generation, as the plants were grown in the greenhouse, and under such conditions the glumes of all plants were white or nearly so. In the  $F_3$  generation the strains were recorded as brown, segregating, or white for glume color. The data are presented in Table 5.

TABLE 5.—Breeding behavior in the  $F_3$  generation for glume color of 107 strains of the Hope  $\times$  Hard Federation wheat cross grown at Bozeman, Mont., in 1929

Breeding behavior for glume color	Number of $F_3$ strains		Deviation
	Obtained	Calculated on 1:2:1 ratio <sup>a</sup>	
Brown.....	29	27	2
Segregating.....	59	53	6
White.....	19	27	8
Total.....	107	107	-----

<sup>a</sup>  $P=0.21$ .

These data reveal a slight shortage of white-glumed strains, although the value  $P=0.21$  indicates a fairly close fit to the 1:2:1 ratio explained by a single genetic-factor difference.

#### KERNEL COLOR

The inheritance of red kernel color was first reported by Biffen (1) as dominant over white in the  $F_1$  generation and as segregating in a 3:1 ratio in the  $F_2$ . Nilsson-Ehle (10) later reported crosses which in the  $F_2$  gave 15:1 and 63:1 ratios of red to white-kerneled plants, proving that two and three genetic factors were present. Similar ratios have since been found by other workers.

In the present study red proved dominant, as usual, in the  $F_1$  generation, and in the  $F_2$  generation all the plants harvested in the greenhouse had red kernels. In the  $F_3$  the strains were described for kernel color as either red or segregating. No true-breeding white-kerneled strains were recovered. The data on segregation are presented in Table 6.

These data reveal the absence of white-kerneled strains and the presence of more true-breeding red than segregating strains. On a basis of three dominant factors for red color, 37 parts are expected to breed true for red, 8 parts to segregate 63:1, 12 parts to segregate 15:1, and 6 parts to segregate 3:1. Thus there are 26 parts segregating to 37 breeding true for red. On this calculated basis the value of  $P=0.34$  indicates a fairly good fit to the 3-factor 37:26:1 ratio.

TABLE 6.—*Breeding behavior in the F<sub>3</sub> generation for kernel color of 107 strains of the Hope × Hard Federation wheat cross grown at Bozeman, Mont., in 1929*

Breeding behavior for kernel color	Number of F <sub>3</sub> strains		Deviation
	Obtained	Calculated on a 37:26:1 ratio <sup>a</sup>	
Red.....	65	62	3
Segregating.....	42	43	1
White.....	0	2	2
Total.....	107	107	-----

<sup>a</sup>  $P=0.34$ .

## AWNEDNESS

Biffen (1) was the first to point out that the awnless condition of a wheat spike is dominant. Other early workers obtained similar results. The beardless or awnless type referred to by these investigators was evidently tip awned, corresponding to class 3, or the awnleted type, of the present study.

Howard and Howard (9) were the first to work with the true awnless wheats, and they reported that two factors were necessary for an interpretation of the inheritance of awns. In similar studies between Kota × Hard Federation crosses or true awnless (class 1) and fully awned (class 5) parents, Clark (4) concluded that two genetic factors could not entirely account for the breeding behavior.

In crosses between awnless (class 1) and awnleted (class 3) wheats, Clark and Hooker (6) have shown that two factors were necessary to explain satisfactorily the segregation of Hard Federation × Marquis crosses.

The inheritance of awnedness was more thoroughly reviewed and further studied by Clark, Florell, and Hooker (5), who classified hybrids into five classes—(1) awnless, (2) apically awnleted, (3) awnleted, (4) short awned, and (5) awned. In crosses between the awnless Bobs and Hard Federation (class 1) parents with the awned Propo (class 5) parent, imperfect dominance of awnlessness was shown as the F<sub>1</sub> (class 2) approached nearer the awnless than the awned parent. The F<sub>2</sub> and F<sub>3</sub> plants were separated into the five classes. The Bobs × Propo cross was less complicated in F<sub>3</sub> than the Hard Federation × Propo cross, the former segregating into 7 breeding groups and the latter into 11. A genetic interpretation of the results for the Bobs × Propo cross was made on the basis of two major (AA and BB) factor pairs. The corrected F<sub>2</sub> for the five classes was found to be close to a 1 : 8 : 4 : 2 : 1 ratio.

The study was continued in the present cross, in which Hope is awned, class 5—, and Hard Federation awnless, class 1+. The plus (+) and minus (−) signs are used to indicate the direction of overlapping. The F<sub>2</sub> plants of the Hope × Hard Federation cross grown in the greenhouse were carefully separated into five awnedness classes as defined by Clark, Florell, and Hooker. The data are shown in Table 7.

The F<sub>1</sub> and the largest number of F<sub>2</sub> plants were of class 2, apically awnleted. These approach more closely the awnless than the awned parent. An imperfect dominance of awnlessness is again shown,

although a larger number of awnleted (class 3) plants in the  $F_2$  was recorded in this cross than in the other crosses studied.

TABLE 7.—Segregation for awnedness in  $F_2$  plants of Hope  $\times$  Hard Federation crosses grown in a greenhouse at the Arlington Experiment Farm, 1928-29

Class	F <sub>2</sub> plants		Class	F <sub>2</sub> plants	
	Num-ber	Per-cent		Num-ber	Per-cent
(1) Awnless.....	10	4.2	(4) Short-awned.....	18	7.6
(2) Apically awnleted.....	104	43.9	(5) Awned.....	28	11.8
(3) Awnleted.....	77	32.5	Total.....	237	100

A total of 107  $F_2$  plants was selected at random for growing in the  $F_3$  generation. The material proved to be rather variable, as it was possible to separate 21 types of segregation. These are shown in Table 8. The modal classes are indicated by italic numbers. Several of these types may not differ inherently from the others. They are arranged in the order of length of awns, and totals are drawn between groups not regarded as inherently different. Variation within the groups may be due either to minor genetic factors or to environment.

TABLE 8.—Breeding behavior of 107  $F_3$  strains of Hope  $\times$  Hard Federation crosses at Bozeman, Mont., 1929

Awnedness class No. —	Number of $F_3$ strains grown from $F_2$ awnedness class No.—					Total	
	1	2	3	4	5		
<i>1</i> .....	4					4	
<i>1, 2</i> .....			1			1	
Total.....							5
<i>1, 2, 3</i> .....	1	2				3	
<i>1, 2</i> .....		1				1	
<i>1, 2, 3</i> .....	1	16				17	
<i>2, 3</i> .....		2				2	
Total.....							23
<i>1, 2, 3, 4</i> .....		2				2	
<i>1, 2, 3, 4, 5</i> .....		4	1			5	
<i>1, 2, 3, 4, 5</i> .....		8	5			13	
<i>2, 3, 4, 5</i> .....		1	3			4	
Total.....							24
<i>2, 3, 4</i> .....		1				1	
<i>3</i> .....			7	1		8	
<i>3, 4</i> .....			3	2		5	
Total.....							14
<i>2, 3, 5</i> .....			4			4	
<i>3, 4, 5</i> .....			5	1		6	
<i>3, 5</i> .....		1	6			7	
Total.....							17
<i>3, 4</i> .....				1		1	
<i>2, 3, 4, 5</i> .....				1		1	
<i>3, 4, 5</i> .....			1	7	9	17	
<i>3, 4, 5</i> .....					1	1	
Total.....							20
<i>5</i> .....					4	4	4
Total.....	6	38	36	13	14		107

\* Modal classes are indicated by italic numbers.

There is a wide range of overlapping among the  $F_2$  awnedness classes. Of the 6 awnless plants grown, 4 bred true and 2 segregated for classes 1, 2, and 3. Of the 14 awned plants grown, 4 bred true and 10 segregated for classes 5, 4, and 3. All the other true-breeding and segregating types are shown with the modal class indicated. There were true-breeding awnleted or class-3 strains but no true-breeding class-2 or class-4 strains.

Allowing for a wide range of overlapping of the classes, the material was separated into seven groups (Tables 8, 9, 10), i. e., (I) strains of class 1, 2, or 3 that have a mode of class-1 plants and breed true or to the limits of classes 1 and 2; (II) strains of class 2 that have a mode of class-1 or class-2 plants and segregate for classes 1, 2, and 3, or two of them; (III) strains that segregate for classes 1, 2, 3, 4, and 5, or at least four of them; (IV) strains of class 3 that breed true or have a mode of class-3 plants and do not contain plants of classes 1 and 5; (V) strains that segregate for classes 3 and 5, or have a mode of class-3 plants with only a few class-2 or class-4 plants; (VI) strains of class 4 that have a mode of class-4 or class-5 plants and segregate for classes from 2 or 3 to 5; and (VII) strains of class 5 that breed true. Table 9 shows the number of plants in each awnedness class in each of the seven groups.

TABLE 9.—Number of  $F_3$  plants in each awnedness class for each  $F_3$  breeding group

F <sub>3</sub> breeding groups	Number of F <sub>3</sub> plants in awnedness class No.—					
	1	2	3	4	5	Total
I.....	245	15				260
II.....	247	655	364			1,266
III.....	80	389	508	203	113	1,293
IV.....		15	731	47		793
V.....		18	639	63	233	953
VI.....		1	242	564	311	1,118
VII.....					218	218
Total.....	572	1,093	2,484	877	875	5,901

In Group I there were 15 plants in 260 that were classified as of class 2. In Group III, which should segregate as in the  $F_2$  generation, there were more class-3 than class-2 plants. In Groups IV, V, and VI there was considerable variation resulting in overlapping.

In Table 10 the percentages obtained in the  $F_2$  generation are corrected upon the breeding behavior of the  $F_3$  strains. For example, of awnless plants (class 1) progenies of 6 of the 10 plants so classified in  $F_2$  were grown and 4, or 66.7 per cent, bred true. This percentage of 4.2 per cent, originally classified as awnless (class 1), is 2.8 per cent. In class 3, the progeny of 1 of the 36 plants grown, or 2.8 per cent of the class, bred as Group I. This percentage of 32.5 per cent is 0.9 per cent. The total of 2.8 and 0.9 per cent gives a corrected percentage of 3.7 awnless, or Group I. Of awned plants (class 5) progenies of 14 of the 28 plants so classified were grown, and of these 4, or 28.6 per cent, bred true. This percentage of the original 11.8 per cent is 3.4 per cent. These 3.7 per cent awnless, and 3.4 per cent awned, approach 6.25 per cent, or one-sixteenth of the total. A closer approach would result by assuming from Table 8 that the variability of both these classes extends to class 3. A poorer approach would result by allowing for no variability or overlapping.

TABLE 10.—Breeding behavior of 107  $F_3$  strains with the original and corrected  $F_2$  classification of 237 plants

F <sub>3</sub> breeding group	Number and percentage of F <sub>3</sub> strains by F <sub>2</sub> class No.--										Total	Corrected F <sub>2</sub> class	Corrected per centage F <sub>2</sub>
	1		2		3		4		5				
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent			
I.....	4	66.7			1	2.8					5	1	3.7
II.....	2	33.3	21	55.3							23	2	25.7
III.....			15	39.5	9	25.0					24	2	25.5
IV.....			1	2.6	10	27.8	3	23.1			14	3	11.9
V.....			1	2.6	15	41.6	1	7.7			17	3	15.2
VI.....					1	2.8	9	69.2	10	71.4	20	4	14.6
VII.....									4	28.6	4	5	3.4
Total.....	6	100.0	38	100.0	36	100.0	13	100.0	14	100.0	107		100.0
Original F <sub>2</sub> :													
Total number.....	10		104		77		18		28		237		
Per cent.....		4.2		43.9		32.5		7.6		11.8			100.0

The results of these studies indicate that a genetic interpretation should be confined to two major factors. With respect to these the formula for the awnless Hard Federation parent would be  $AABB$ , and that for the awned Hope parent would be  $aabb$ . On this basis the phenotypic class, general breeding behavior, and genotypic groups may be indicated as follows:

## PHENOTYPIC CLASS, BREEDING BEHAVIOR, AND GENOTYPIC GROUP

Class 1, Awnless:			Group
1 $AABB$ .....	Breeding true.....		I
Class 2, Apically awnleted:			
2 $AABb$ .....	Segregating for classes 1, 2, and 3.....		II
2 $AaBB$ .....			
4 $AaBb$ .....	Segregating as in F <sub>2</sub> for classes 1, 2, 3, 4, and 5.....		III
8 $Aabb$ .....			
Class 3, Awnleted:			
1 $AAbb$ .....	Breeding true.....		IV
1 $aaBB$ .....			
2 $Aabb$ .....	Segregating for classes 3 and 5.....		V
4 $Aabb$ .....			
Class 4, Short-awned:			
2 $aaBb$ .....	Segregating for classes 3, 4, and 5.....		VI
Class 5, Awned:			
1 $aabb$ .....	Breeding true.....		VII

The goodness of fit is shown in Table 11, based on calculated numbers for two genetic factors on both 5 phenotypic classes and 7 genotypic groups. The agreement is very good but there is a slight shortage of both awnless and awned strains. The 1:8:4:2:1 phenotypic ratio is the essential explanation of inheritance in the 5 awnedness classes. The value  $P=0.27$  for the genotypic ratio indicates that a worse fit, due to chance alone, might be expected 27 times in 100 trials. It would seem, therefore, that any theory assuming two major genetic-factor differences should satisfactorily explain the inheritance of awnedness in this cross. Although it is recognized that some of the variability observed may have been caused by minor factors, it has been impossible to prove the interaction of an additional genetic factor or factors because of the effects of uncontrolled variation caused by environment.

TABLE 11.—*Goodness of fit determined on a 2-factor basis for 237 F<sub>2</sub> plants corrected on the basis of the breeding behavior of 107 F<sub>3</sub> strains*

F <sub>2</sub> genotypic groups	Corrected F <sub>2</sub> class	Number of F <sub>2</sub> plants			
		5 phenotypic classes		7 genotypic groups	
		Obtained	Calculated <sup>a</sup>	Obtained	Calculated <sup>b</sup>
I.....	1	9	15	9	15
II.....	2	122	118	61	59
III.....	2			61	59
IV.....	3	64	59	28	29
V.....	3			36	30
VI.....	4	34	30	34	30
VII.....	5	8	15	8	15

<sup>a</sup>  $\chi^2 = 6.76$ ;  $P = 0.15$ .<sup>b</sup>  $\chi^2 = 7.57$ ;  $P = 0.27$ .

No consistent or important relation was found between the seven genotypic awnedness groups and the percentage of bunt or yield per plant, as determined by correlation ratios.

#### SUMMARY

The parent reaction to bunt, or stinking smut, and the segregation as shown by the average of F<sub>3</sub> hybrid strains for three crosses, Hope × Marquis, Hope × Ceres, and Hope × Hard Federation, was studied. Hope was shown to be strongly resistant and Marquis weakly resistant, averaging 2.1 and 44.2 per cent of bunted plants, respectively. Ceres was found weakly susceptible and Hard Federation strongly susceptible with an average of 64.2 and 81.7 per cent of bunted plants, respectively. The Hope × Hard Federation cross showed a strong tendency for dominance or a preponderance of susceptibility. No F<sub>3</sub> strain was found within the limits of the Hope parent, indicating the presence of several genetic factors. In the Hope × Ceres cross an intermediate inheritance was indicated, with a normal curve between the parents but with considerably less than one-fourth within the limits of each parent. In the Hope × Marquis cross there was a tendency toward a dominance of the stronger resistance of the Hope parent, with only 8.7 per cent within the limits of the weak resistance of the Marquis parent. The results from the three crosses indicate that the stronger the degree of resistance involved in the hybrids the less complicated is the inheritance, with an increasing tendency away from an imperfect dominance of susceptibility toward an imperfect dominance of strong resistance.

In the Hope × Hard Federation populations the percentage of bunt was about one-fourth the percentage of infected plants. The negative correlation  $r = -0.554 \pm 0.045$  was found between percentage of bunt and average yield per plant.

The correlation between percentage of bunt and percentage loss in yield was found to be  $r = +0.501 \pm 0.049$ . The regression of percentage loss in yield on percentage of bunt is shown. An increase of 5 per cent in bunt caused a decrease of about 4 per cent in yield.

Glume color was shown to be controlled by a single genetic-factor difference, a 1:2:1 ratio for brown, segregating, and white having been obtained.



Kernel color was found to be controlled by at least three genetic factors, with a good fit to the theoretical 37:26:1 ratio for red, segregating, and white.

The expression of awnedness was shown to be rather variable, but by grouping different segregating types a good fit was shown for five phenotypic classes and for seven genotypic groups. Two major factors are clearly shown, with a 1:8:4:2:1 phenotypic ratio for five awnedness classes. No important relation was found between awnedness on the one hand and bunt or yield on the other.

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# RHABDITIS LAMBDIENSIS, A NEMATODE POSSIBLY ACTING AS A DISEASE AGENT IN MUSHROOM BEDS<sup>1</sup>

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## INTRODUCTION

Recently, Prof. L. Haseman, of the College of Agriculture, University of Missouri, submitted larval and adult nemas that according to his observations had been destructive in mushroom beds in Leeds, Mo. Professor Haseman writes:

Two or three weeks before the complaint was received I saw the mushroom cellar and it was in perfect condition as regards the development of the mushrooms and the complete absence of insect pests and other enemies of mushrooms. All of a sudden, apparently due to overwatering, a portion of the house showed the work of these little worms. They appeared on the beds as well as on the top of the mushroom caps. A dozen or so worms of varying sizes could be found in a little decaying pit on the top of the cap and from day to day the size of these decaying pits increased, and I take it multiplication or development of the worms continued. Eventually the entire mushroom would decay.

According to the grower, some of the beds in one cellar were practically ruined. The present paper reports the results of an investigation of this problem.

## RHABDITIS LAMBDIENSIS AS A DISEASE AGENT

The "worms" submitted by Professor Haseman proved to be nemas of the species *Rhabditis lamdbiensis* Maupas. *Rhabditis* species are usually saprophytic or saprozoic, although a few are known to parasitize man and animals and many occur in diseased plants. *R. lamdbiensis* has been reported under the name *R. monhystera* Bütschli<sup>3</sup> (1) on decaying leaves of banana plants in Fiji, and (2) from fresh and living grass and on celery in Australia,<sup>4</sup> and under the name *R. lamdbiensis* (3) from moist soil in three localities in French North Africa.<sup>5</sup> It is further known to have occurred in decaying plant material in Washington, D. C., and in Salem, Utah.<sup>6</sup>

## POSSIBLE RELATIONSHIP BETWEEN RHABDITIS AND THE PRESENT MUSHROOM DISEASE

The association of *Rhabditis lamdbiensis* with mushrooms may be interpreted in several ways. A saprophytic organism may live (1) on the contents of the cells of a dead plant (more or less decomposed),

<sup>1</sup> Received for publication Aug. 10, 1932; issued April, 1933.

<sup>2</sup> For the literature references citing the works of Falconer and of Elliott (footnotes 7 and 8) the writer is indebted to Neil E. Stevens and E. B. Lambert, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>3</sup> COBB, N. A. NEMATODES, MOSTLY AUSTRALIAN AND FIJIAN. In Fletcher, J. J. The Macleay Memorial Volume, p. 252-308. Sydney. Reprinted in N. S. Wales Dept. Agr., Misc. Pub. 13: 29. 1893.

<sup>4</sup> COBB, N. A. Op. cit.

<sup>5</sup> MAUPAS, E. ESSAIS D'HYBRIDATION CHEZ DES NEMATODES. Bul. Biol. France et Belg. 52: 466-498, illus. 1919. In connection with the taxonomic position of the present species, compare also:

MAN, J. G. DE. DAS MÄNNCHEN DER RHABDITIS MONHYSTERA BÜTSCHLI. Zool. Anz. 70: 51-57, illus. 1926.

<sup>6</sup> Oral communications by B. G. Chitwood, of the Zoological Division, Bureau of Animal Industry, and G. Thorne, of the Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture.

(2) on the substance of the cell walls, (3) on the bacteria or fungi associated with decay, or (4) on waste material due to decay-producing organisms.

In view of the fact that some *Rhabditis* species are known to feed exclusively on fungi, bacteria, or gelatinous products of bacteria, it seemed possible that this might be true of *Rhabditis lambdiensis* also. To determine this, the contents of the intestinal tract of a number of specimens were carefully examined. Agglomerations or balls of bacteria were found in the pharynx and in the anterior portions of the intestine. This led to the conclusion that *R. lambdiensis*, if not the primary cause of the present mushroom disease, may act as a carrier of pathogenic bacteria. In 1910 William Falconer<sup>7</sup> mentioned a mushroom disease identical with the one here dealt with, or at least very similar to it. He calls it "black spot," and considers the cause to be eelworms (*Anguillulae*). He explains that brown spots, streaks, or freckles appear on the top of the mushroom caps, increasing in distinctness and breadth with age. The presence of nemas in all these spots led Falconer and those whom he consulted to believe that these *Anguillulae* were the cause of the disease. The blackspot or brown-spot disease of mushrooms was later recognized as of bacterial origin by various authorities.<sup>8</sup> It therefore seems that the pathogenic agent, *Bacterium tolaasi* (Paine),<sup>9</sup> may act alone or together with nemas. It is impossible from Falconer's account to recognize the nematode species involved. The term "*Anguillulae*" applied by him undoubtedly means nemas, or eelworms, in general, and is not a plural of the generic name *Anguillula*, which includes only the vinegar eel and a few related forms. It is therefore possible that Falconer's eelworms were similar to *R. lambdiensis* or identical with it.

The statement of Falconer and his associates, as well as Professor Haseman's observations on the relationship of the nemas to the disease, are undoubtedly pertinent. The nema, however, is the secondary agent. Nevertheless, in the present case it almost equals in importance the primary cause of the disease. It has been repeatedly pointed out that saprophytic and saprozoic nemas are important agents in the distribution of bacterial and fungous diseases of plants. It is known that these nemas travel readily on moist surfaces. Since mushroom beds must be sprinkled regularly, there is an opportunity for the *Rhabditis* to spread quickly, carrying along the pathogenic bacteria attached to the body surface or the oral opening, in the pharynx (the contents of which are sometimes emitted again), or in the intestine, from which they are discharged with the feces.

#### MORPHOLOGY OF RHABDITIS LAMBDIENSIS

It will be seen that the present description differs somewhat from that of Maupas<sup>10</sup> and also from that of Cobb<sup>11</sup> for *Rhabditis monohystera* (declared by Maupas to be a synonym of *R. lambdiensis*) from Fiji and Australia, especially in regard to the structure of the lip region and the arrangement of the bursal ribs. It is, however, thought that these slight differences may represent individual variations or difficulties of observation, especially of the lip region.

<sup>7</sup> FALCONER, W. MUSHROOMS: HOW TO GROW THEM: A PRACTICAL TREATISE ON MUSHROOM CULTURE FOR PROFIT AND PLEASURE. p. 124-126. New York. 1910.

<sup>8</sup> ELLIOTT, C. MANUAL OF BACTERIAL PLANT PATHOGENS. p. 227. Baltimore. 1930.

<sup>9</sup> ELLIOTT, C. Op. cit., p. 226-227.

<sup>10</sup> MAUPAS, E. Op. cit.

<sup>11</sup> COBB, N. A. Op. cit.

## THE CUTICLE

The cuticle is rather complicated because the annules are reticulated (areolated) and have points, granules, and ridges that show considerable variability, especially in different regions of the body. Furthermore, these cuticular structures seem to vary in individuals. The regular reticulation, for example, extends much farther back in some specimens than in others. Immediately posterior to the lip region, the cuticle is simply annulated. The reticulation begins in the latitude of the anterior end of the esophagus. Here one sees the beginning of an areolation due to the appearance of small "shields" with rounded posterior edges, seen with remarkable plainness when the animal moves. Soon quadrangular areolae appear (figs. 1, F, and 2, A-H) accompanied by one or two points or rods; still farther back the areolation is not apparent but may reappear toward the tail end. However, transverse series of points may be seen all along the body, sometimes distributed rather profusely and irregularly. (Fig. 2, A-H.) On the lateral surface of the anterior portion of the body, at least, longitudinal crenate wings can be seen in considerable numbers. From optical profile views it is concluded that the points mentioned above are not surface structures but are located under the first cuticular layer. (Fig. 2, B, D, F, and H.)

## DEIRIDS AND PHASMIDS

A deirid or cervical papilla was seen in the latitude of the nerve ring. As it did not protrude, it was recognized only when seen in profile. (Fig. 2, K.) On the male occurs a similar papilla, about three spicula lengths in front of the anus. (Fig. 2, L.) This was at first thought to be one of a series of lateral papillae, such as are found in other nemic forms, but no such series was located. Phasmids were not seen, although they were probably present. Maupas's drawing could be interpreted as having them.

## THE LIP REGION AND ITS SENSE ORGANS

All the writer's specimens had 6 lips, each with somewhat angular outlines. (Fig. 1, A, B, and F.) Undoubtedly Maupas was mistaken in assuming the presence of only 3 lips. His drawing of the frontal view of the head shows 3 groups of 2 lips. In the present case and as here figured, no grouping was seen. Contrary to Maupas's<sup>12</sup> account and contrary also to Cobb's<sup>13</sup> descriptions of his *Rhabditis monhystera*, in which mention is made of only 1 setalike papilla for each lip, 3 papillae were seen on each submedial lip and 2 on each lateral lip. In front view, each submedial lip showed a group of 2 papillae belonging to an outer circle and 1 papilla belonging to an inner circle. The lateral lips, however, have apparently only 2 papillae, an inner and an outer one, both rather indistinct. The papillae are not setiform, but simply minute elevations. Inasmuch as Maupas's drawings show setiform papillae there is a possibility that the present form is different.

The lips are separated by rather wide and apparently deep furrows which lead outward to rounded excisions (fig. 1, B), intensifying the angular appearance of the lips. The amphids, not hitherto described for the present species, seem papillate if superficially seen. Close

<sup>12</sup> MAUPAS, E. Op. cit.<sup>13</sup> COBB, N. A. Op. cit.

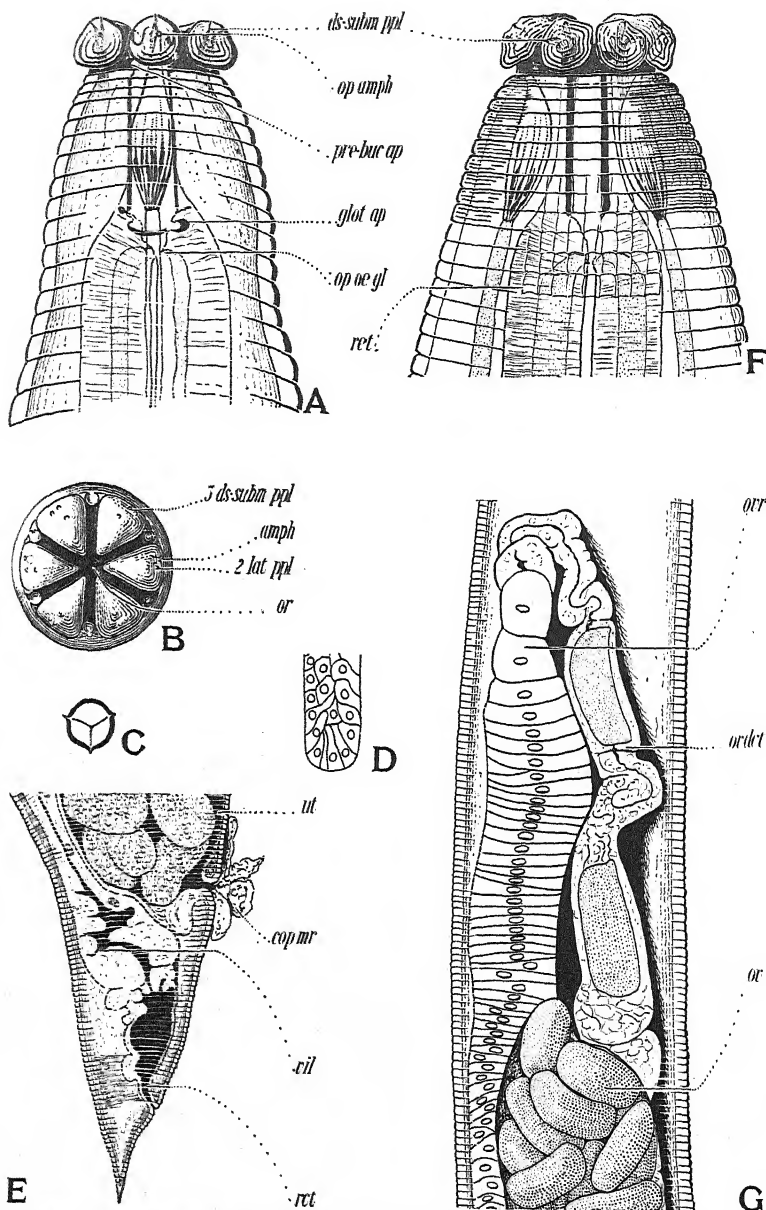


FIGURE 1.—*Rhabditis lamdiensis*, female: A, Lateral view of head end: *ds-subm ppl*, dorsosubmedial papilla; *glot ap*, glottoid apparatus; *op amph*, opening of the amphid; *op oe gl*, opening of an esophageal gland; *pre-buc ap*, prebuccal apparatus.  $\times$  about 1,600. B, Front view of head: *amph*, amphidial opening; *3 ds-subm ppl*, 3 dorsosubmedial papillae; *2 lat ppl*, 2 lateral papillae, *or* mouth opening.  $\times$  about 1,600. C, Optical cross section through buccal cavity. Free-hand sketch. D, Blind end of ovary. Free-hand sketch. E, Posterior end of body: *cop mr*, copulation marks; *rct*, rectum; *ut*, uterus; *cil*, villilike process.  $\times$  about 250. F, Dorsosventral view of head end: *ds-subm ppl*, dorsosubmedial papilla; *ret*, reticulation of the cuticle.  $\times$  about 1,600. G, Region of the anterior oviduct: *or*, egg in uterus; *ovidt*, oviduct; *orr*, ovary.  $\times$  about 250

observation shows that they open slightly dorsad from the outer lateral papillae. Figure 1, A and F, shows side and profile views of them. The so-called amphidial pouch (sensilla) is apparently gourd-shaped or flask-shaped, extending inward to nearly the beginning of the esophageal region and widening in the postlabial portion. A number of nerve terminals were faintly discernible inside the pouch. Posteriorly this structure connects with its nerve, which by close observation could be followed nearly to the nerve ring.

#### THE ALIMENTARY TRACT

The vestibule consists of a cuticular apparatus at the base of the lip region, which can be seen best on living specimens, preferably in side

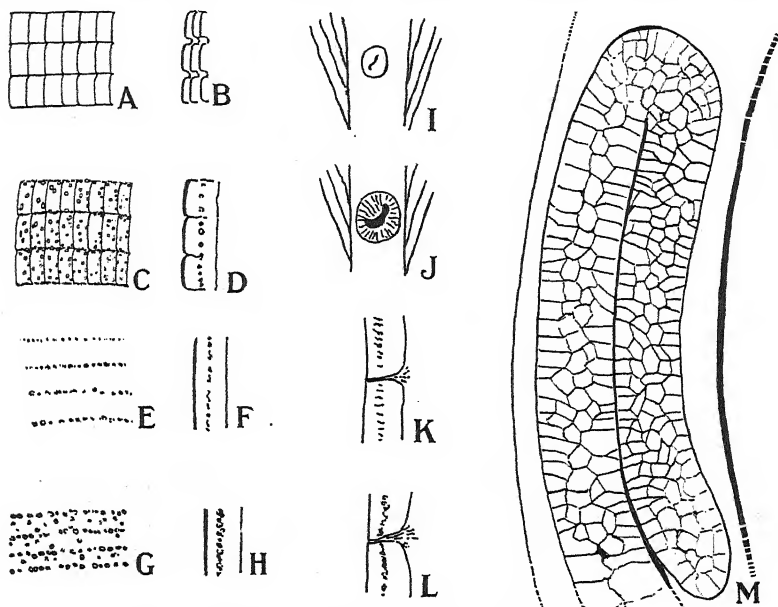


FIGURE 2.—*Rhabditis lambdiensis*: A, Surface view of reticulation of cuticle, anterior to median bulb; B, profile view of A; C, surface view of reticulation of cuticle, near excretory pore; D, profile view of C; E, surface view of reticulation of cuticle, middle region of the body; F, profile view of E; G, surface view of reticulation of cuticle, in front of spicula; H, profile view of G; I, surface view of excretory pore; J, deeper focus on excretory pore; K, deirid, profile view; L, lateral papilla on male, three spicula lengths in front of anus; M, blind end of testis. A-L, Free-hand sketches; M,  $\times$  about 370

view; its parts closely resemble teeth, especially when moving. However, its function may be mainly that of supporting the exceedingly mobile lip region. Doubtless in structure and function this prebuccal apparatus is similar to, if not homologous with, the so-called glottoid apparatus behind the buccal cavity. Both are very mobile sections separating more rigid parts. The walls of the buccal cavity are not so thick as in many other species of *Rhabditis*. There is an encircling ring at the beginning of the last fourth of the cavity and another at the end of the first fourth. These rings probably are markings, the former produced by muscle attachments and the latter by the attachment of the esophagus.

The esophagus looks like that of other *Rhabditis* species. There are 3 esophageal or salivary glands, 1 dorsal and 2 ventrosubmedial.

The nucleus of each is located in the cardiac bulb. A narrow canal with slightly thickened walls leads from each gland anteriorad through the isthmus; as the canals reach the oval medial bulb the thick lining disappears, and they seem to widen to a cylindrical granulated portion traversing this bulb and connecting anteriorad with a canal of thickened walls which empties into the esophageal lumen a short distance behind

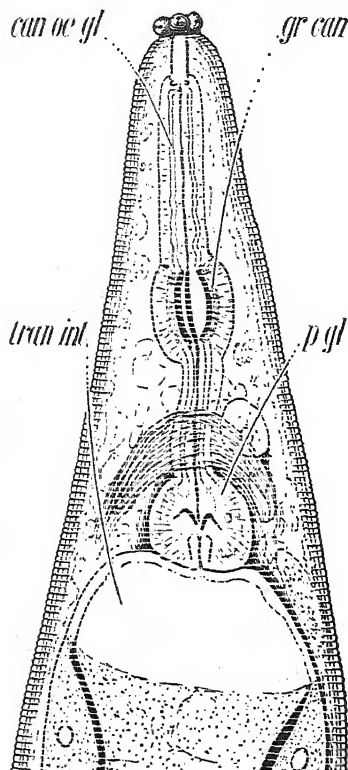


FIGURE 3.—*Rhabditis lamdiensis*, anterior portion of a female: *can oe gl*, canal leading from esophageal or salivary glands; *gr can*, cylindrical granulated portion of canal; *p gl*, position of the nuclei of esophageal or salivary glands; *tran int*, transparent portion of intestinal cells.  $\times$  about 370

the glottoid apparatus. It appeared at first as if the 3 glands emptied into the esophagus where the cuticularized canals left the isthmus and entered the medial bulb; but a more detailed study proved that the granular portions in the medial bulb were apparently connected with the canals.

Six cells form the circumference of the intestine. The initial portion of the intestine (fig. 3) is lighter colored and much more transparent than the rest, as the cells in that region are thinner than those posteriorad. The intestinal lumen in the prerectal portion is highly sinuate, the individual cells often forming villilike processes extending into the intestinal cavity. A "Stäbchensaum" is well developed along the entire intestine. Proximad the rectum has the usual three rectal cells.

#### THE EXCRETORY SYSTEM

Maupas described the excretory system of the present form as being of the H type, consisting of two anterior and two posterior lateral branches. The observations of the writer seem to confirm this; the canals, however, were seen only a short distance and are sinuate. A ventral view of the common outlet showed what seem to be radial muscular fibers around its proximal portion (fig. 2, J), where it is somewhat wider than at its intersection with

the cuticle. It is possible that this may be the portion that serves as the "pulsating" vesicle seen in certain other species of *Rhabditis*.

#### THE FEMALE SEXUAL APPARATUS

The female sexual apparatus is of typical prodelphic character. There is practically no sign of a posterior uterine branch; the anterior one, however, is very large and in adult females is filled with numerous eggs which sometimes contain fairly advanced embryos. There is a distinct oviduct (fig. 1, G); its end portion is reflexed and connects with the ovary, which in some instances was seen to extend back



almost to the vulva. The end of the ovary is shown in Figure 1, D; no differentiated terminal cell was seen. Some of the females exhibited so-called copulation marks, i. e., the vulvar opening was covered with a hardened brownish mass, the remains of a cement apparently produced by the male during copulation.

#### THE MALE SEXUAL APPARATUS

The male genital system has a reflexed testis similar in structure to the ovary. The copulatory apparatus is highly complex because of a

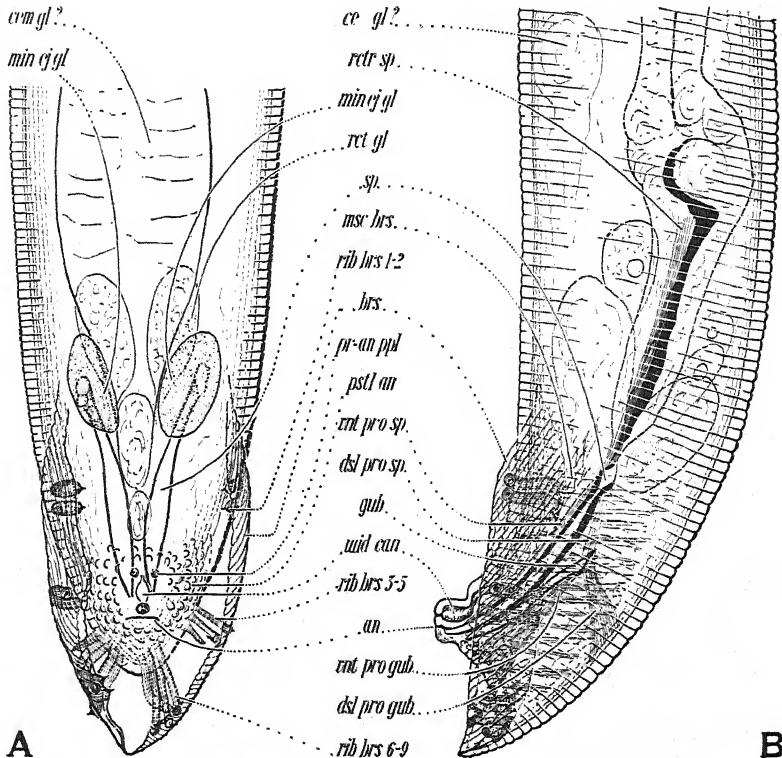


FIGURE 4.—*Rhabditis lambdiensis*, posterior portion of male: A, Ventral view; B, lateral view. *an.*, anus; *hrs.*, bursa; *cem gl?*, cement gland (?); *dsl pro gub.*, dorsal protrudor muscle of gubernaculum; *dsl pro sp.*, dorsal protrudor muscle of spicula; *gub.*, gubernaculum; *min ej gl.*, small ejaculatory gland (?); *msc hrs.*, muscles of bursa; *pr-an ppl.*, preanal papilla (?); *pstl an.*, pustules surrounding anal region; *ret gl.*, rectal gland (?); *retr sp.*, retractor muscles of spicula; *rib hrs 1-2*, ribs of bursa 1-2; *rib hrs 3-5*, ribs of bursa 3-5; *rib hrs 6-9*, ribs of bursa 6-9; *sp.*, spicula; *ent pro gub.*, ventral protrudor muscle of gubernaculum; *ent pro sp.*, ventral protrudor muscle of spicula; *wid can.*, widening of outlet canal. X about 720

number of accessory organs. There is a pair of almost straight spicula (fig. 4, A and B) slightly cephalated at the inner end, the whole somewhat resembling the blade of a knife. A single gubernaculum was seen, similar in shape to the spicula, but only two-thirds as long. (Fig. 4, B.) The following muscles were connected with this spicular apparatus: (1) A long retractor muscle attached to the inner end and running obliquely forward to the dorsolateral body wall, (2) a ventral protrudor muscle attached also to the inner end of the spicula and to the ventral body wall in front of the anus, (3) a dorsal protrudor muscle connecting the inner spicula ends with the proximal end of the gubernaculum,

(4) a ventral protruder gubernaculi connecting the inner end of the gubernaculum and the ventral body wall back of the anus, and (5) a dorsal protruder gubernaculi attached to the inner end of the gubernaculum and to the dorsal body wall.

As shown in Figure 4, B, there is also a series of so-called bursal muscles present, extending from near the anterior end of the bursa to the middle of the tail. The bursa has very typical characters. It begins slightly in front of the spicula and surrounds the tail completely. (Fig. 4, A and B.) Its surface structure is much like the cuticle, although not so regular. Figure 4, A and B, shows the striation of such a bursal wing. The ribs as seen in all the present material have a slightly different arrangement from that figured by Maupas<sup>14</sup> and that sketched by Cobb<sup>15</sup> for his Australian specimens. There were probably 9 ribs in each of the specimens. Cobb mentions only 8 for his specimens, although his drawings show that there might have been 9. However, one of the group of 5 close to the tail end is very difficult to see and perhaps escaped the observer's notice. There is a pair of ribs in the latitude of the middle of the spicula. This pair seems to be constant and has been reported at the same place by all observers. Both ribs reach the edge of the bursa and each has a distinct terminal papilla. The second group of ribs is about in the latitude of the anus or slightly in front of it. According to Maupas's description, there are also 2 ribs in this group; this agrees with observations of the writer. In one of the specimens (fig. 4, A), however, 3 ribs were seen in this group in the left bursal wing. This is very significant because the forms described by Cobb from Australia seemed to have this as the normal number. It is therefore concluded that this species varies in this character. The last group, if normal, consists of 5 ribs which are close to the tail end. Maupas draws these ribs as slightly spaced, but in the writer's material they were close together. (Fig. 4, A.) Possibly there is some variation also in this regard. One rib, which seems to fluctuate somewhat between the second and the third group, apparently is only half as long as the others and ends on the outer surface of the bursa. Moreover, this rib differs from the others in tapering distally. The anterior rib of the second group likewise ends on the outer surface of the bursa, with a distinct point; the posterior one, however, extends to the rim of the bursal wing. The 4 posterior ribs of the third group likewise differ in this respect; the first one extends to the bursal rim, the second does not quite reach it and is seemingly turned outward, the third reaches the bursal rim, and the fourth ends on the outer surface apparently about halfway to the rim. Each of these 4 ribs ends with what seems to be a pointed papilla.

The anal region of the male, especially the part in front of the anus, protrudes in a most remarkable way (fig. 4, B); a broad elevation is formed through which penetrates the outlet of what seems to be the cement gland. In ventral as well as profile view one observes an ampullalike widening of the outleading canal. (Fig. 4, A and B.) It is, however, difficult to connect this with the cement gland itself, which seems to be a large body located ventrad of the intestine and ejaculatory duct in front of the spicula. It is rather strange that this gland should be single in the present species, whereas it is paired in

<sup>14</sup> MAUPAS, E. Op. cit.

<sup>15</sup> COBB, N. A. Op. cit.

other species of *Rhabditis*. Chitwood<sup>16</sup> points out that in some species of *Rhabditis* there exist still other glands in this region which in structure can not be differentiated from the cement glands and that it is not even established which of the glands produce the cement. He therefore designates the large pair (the "cement glands" of other authors) as "large ejaculatory glands" and the others as "small ejaculatory glands."

In the present species, two small ejaculatory glands are present (fig. 4, A) in addition to the triple rectal glands.

At present *Rhabditis lambdiensis* is the only species that has a preanal outlet for the cement gland opening on top of a large papillate elevation.

Still another rather remarkable character typical of this species is the presence of a considerable number of small round pustules arranged in circular series surrounding the anal opening.

In front of the papillate elevation previously mentioned is what seems to be a pair of preanal papillae, structures not hitherto seen in *Rhabditis*. They are rather obscure and are seen distinctly only in a ventral view. These papillae may be the openings of the two small ejaculatory glands.

#### SUMMARY

*Rhabditis lambdiensis* Maupas is described as a probable carrier and distributor of a pathogenic agent (*Bacterium tolaasi*) in mushroom beds and cellars. Its relationship to the so-called black-spot or brown-spot disease of cultivated mushrooms is discussed. A detailed description of the nema is given, including new data on the morphology of this species and on that of the genus in general.

<sup>16</sup> CHITWOOD, B. G. STUDIES ON SOME PHYSIOLOGICAL FUNCTIONS AND MORPHOLOGICAL CHARACTERS OF RHABDITIS (RHABDITIDAE, NEMATODES). Jour. Morph. and Physiol. 49: 251-275, illus. 1930.



# ELIMINATION OF NATURAL MORTALITY AS A FACTOR IN DETERMINING THE EFFECTIVENESS OF HYDRO- CYANIC ACID GAS ON THE CALIFORNIA RED SCALE<sup>1</sup>

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## INTRODUCTION

In tests on the fumigation of the California red scale (*Chrysomphalus aurantii* Mask.) with hydrocyanic acid gas, natural mortality as ordinarily considered has ranged from 1 to 71 per cent. This has introduced so large an error in experimental tests as to render precise results almost impossible. Therefore, an attempt has been made to establish qualitative tests, based on such phenomena as changes in color and conformation, by which the scales killed by treatment could be distinguished from those that died naturally. Though a complete separation has not been possible, the number of dead scales occurring on untreated, or check, lemons which are indistinguishable from those killed on recently fumigated fruit have proved to be few. However, it has been found that the scales dying from natural causes bear a nearly constant ratio to the number of living scales plus the number of dying scales. This paper is concerned with the amount of fluctuation that one may expect in natural mortality, as defined by these new criteria.

When the California red scale is killed by treatment with hydrocyanic acid gas, it becomes darker in color and then gradually dries out. Scales dying naturally go through a similar change. An attempt was made to determine what factors control the numbers of these dying scales. To simplify the problem, it was decided to use only lemon fruits, and to make counts exclusively of the female in the later periods of her development. At this time the female is relatively large and bodily changes can be noted with less chance of error than is possible during the earlier periods of her development. She is also more resistant to hydrocyanic acid gas. The stages used were the fully developed second stage, the gray adult, and the mature adult.<sup>2</sup> On dying, the scales in these three stages first grow darker. Color changes are practically completed before the drying-out process begins. The color of the insect during the fully developed second stage becomes a dull brown to a dark reddish brown, that of the gray adult a dull gray to almost black, that of the mature adult a dirty reddish to blackish red brown. In drying out, the individuals become increasingly viscous, as can be recognized by probing but by no other means, so far as the writers know, up to the time they are nearly dry. Shriveling may or may not accompany the drying-out process.

<sup>1</sup> Received for publication June 3, 1932; issued April, 1933.

<sup>2</sup> The term "fully developed second stage" is applied to that part of the second stage from the time the individual has reached approximately its largest diameter and is becoming shiny, but before the derm has thickened, up to ecdysis; "gray adult" is applied to that part of the third or adult stage from the time of ecdysis until the individual has reached approximately its largest diameter and is becoming shiny and beginning to darken; "mature adult" is applied to the adult stage beyond the gray adult one.

The experimental data show that the ratio of dying to living plus dying scales<sup>3</sup> of the total number in the three periods from untreated fruits in fair condition varies but little, even though the factors involved may differ. This is not true for the ratio of living or of dead scales to the total scale count, where those already dead and dried out are also included. The ratio of dying scales to living plus dying scales on untreated fruits shows so little variation that by taking the weighted mean of a moderate number of samples a satisfactory value for expressing mortality can be obtained. This value may then be used in eliminating the natural mortality from counts on treated samples. The terms "mortality" and "natural mortality" occurring hereafter, when used without explanation, refer to the ratio of dying scales to living plus dying scales present, expressed as a percentage.

#### STABILITY OF MORTALITY VALUES ON UNTREATED CHECKS

To be of broad applicability for experimental tests, the value for expressing natural mortality should be satisfactorily stable under a variety of conditions encountered in practice. The writers have found that the following factors do not definitely influence this value as obtained by their method: Different locations on the same tree; corresponding locations on different trees; different locations on different trees; size of fruit (samples<sup>4</sup> ranged from 1½ to more than 2 inches long); severity of infestation (single samples ranged in total counts from 16 to 600 scales); protection by overhanging foliage (records were from a grove rather sparsely foliated); time of year (December 3, 1930, to January 12, 1931, for one grove, and January 22 to May 21, 1931, for another); and time and conditions of storage after collection (from none to 50 days under temperature conditions, all approximately uniform, ranging from 13° to 26° C.). As for fruits of varying conditions, counts from those scales that are beginning to dry out noticeably still show the same general mortality at first, but eventually the mortality rises rapidly. In comparing mature or yellow fruits of good condition with immature or green fruits of like condition, the writers have observed a slightly higher mortality for the yellow fruits in the average of a considerable number of counts. The above-mentioned conclusions have been drawn from a total count of almost 25,000 scales, where all factors except the one under investigation were made as nearly alike as possible.

To give an idea of the stability of the mortality values obtained by the writer's method as compared with the usual practice of obtaining the net mortality of treated material from the ratio of live scales to the total number of scales on checks, two frequency distributions have been plotted. (Fig. 1.) The data were obtained from counts on 63 lemons, all green or immature, in good condition and collected from a single grove during two periods in 1931, that is, from January 22 to February 20 in the winter and from May 8 to 21 in the spring. The collections in the winter period were stored from 3 to 21 days at 13° C., those in the spring period were not stored. Counts of the latter were made on the day the samples were collected or on the following day. The abscissa of the chart gives, in terms of percentage, the ratio of living to living plus dying

<sup>3</sup> All scales discolored or in process of drying out are referred to as dying. Living scales denote live scales exclusive of those dying.

<sup>4</sup> In this paper a sample refers to an individual lemon fruit.

scales obtained from the formula  $\frac{A}{A+Dy}$ , and the ratio of living to total number of scales (excluding empty shells) obtained from the formula  $\frac{A}{A+Dy+Dr}$ , in which  $A$  refers to living scales,  $Dy$  to dying scales, and  $Dr$  to dead, dried scales. The percentage of mortality, expressed by the formula  $\frac{Dy}{A+Dy}$ , is, of course, obtained by subtracting the value for  $\frac{A}{A+Dy}$  from 100 per cent. The abscissa is

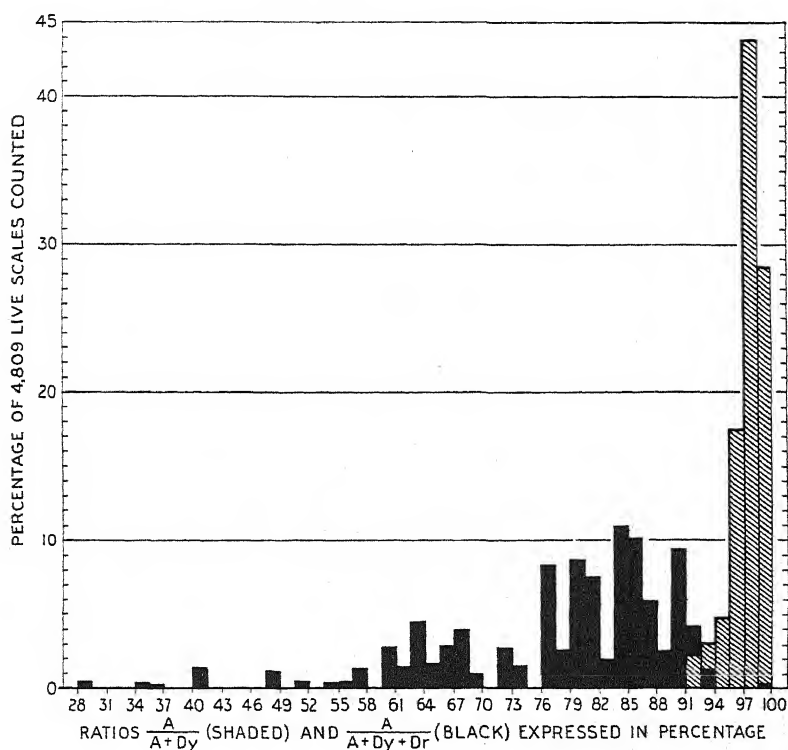


FIGURE 1.—Comparison of variability of the ratio of living scales to living plus dying scales ( $\frac{A}{A+Dy}$ ) and the ratio of living scales to the total number of scales ( $\frac{A}{A+Dy+Dr}$ ) from the same counts

divided into 1.5 per cent intervals. The numbers of live scales on each sample which fell in each 1.5 per cent interval on the abscissa were totaled and the ratio of this total to the total count of 4,809 live individuals, expressed as a percentage, was placed in the appropriate interval on the abscissa and read from the scale on the ordinate. This was done both for the ratio of living to living plus dying scales (shaded columns) and for the ratio of living to living plus dying plus dead and dried scales (black columns). It is seen that the range of mortality obtained by the writers' method is narrow (0 to 9 per cent), whereas that obtained by the usual method is so wide as to introduce a real error in its use.

TABLE 1.—Comparison of variability of the ratio of living scales to living plus dying scales  $\left(\frac{A}{A+Dy+Dr}\right)$  and the ratio of living scales to the total number of scales  $\left(\frac{A}{A+Dy+Dr}\right)$  from counts on fruits with the following factors involved: Grove from which collected, period of collection, storage temperature, and condition of fruit

Grove	Period of collection	Group No.	Fruits used	Storage temperature	Condition of fruit	Scales				Ratios		
						A	Dy	Dr	A+Dy+Dr	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
						Number	Number	Number	Number	Per cent	Per cent	Per cent
A	Jan. 22 to Feb. 20, 1931	1	20	° C.	Good (green)	2,793	69	576	3,438	97.6	2.4	81.2
	Mar. 8-21, 1931	2	43	(*)	do	2,016	51	882	2,949	97.5	2.5	68.4
	Jan. 22 to Feb. 20, 1931	3	5	13	Good (yellow)	1,284	64	360	1,708	95.3	4.7	75.2
	Jan. 22, 1931	4	4	13	Drying	931	28	224	1,183	97.1	2.9	78.7
	May 21, 1931	5	4	(*)	do	541	17	591	1,149	97.0	3.0	47.1
	Jan. 22 to Feb. 20, 1931	6	3	26	Good (green)	680	14	117	811	98.0	2.0	85.8
	Mar. 30, 1931	7	33	13, 17, 20, 23, 26	Good (green or yellow or partly both)	2,062	65	1,776	3,963	95.7	4.3	52.8
B	Dec. 3, 1930, to Jan. 12, 1931	8	47	10	Good (green)	3,898	217	1,807	5,922	94.7	5.3	65.8
C	Jan. 20, 1931	9	13	13	Good (yellow)	861	49	912	1,825	94.6	5.4	47.3
		10	10	26	Good (green)	1,088	50	488	1,636	95.6	4.4	67.1
		11	3	13	Good (yellow)	184	8	21	213	95.8	4.2	86.4

\* Counted promptly after collection.



In Table 1 a series of total counts is presented to show the relation of the mortality ratios to date of collection, temperature at which the fruit was stored (if the counts were not made promptly after collection), and condition of fruit, in three widely separated groves in the Whittier (Calif.) district. Considering the data from grove A, it is seen that counts of fruits collected between January 22 and February 20 and stored from 2 to 10 days in a relatively cold medium (Group 1) show practically the same mortality as counts from fruits collected 3 months later and not stored (Group 2); that counts on fruits stored for about the same length of time in a distinctly warmer environment (Group 6) show approximately the same mortality as those stored at the lower temperature (Group 1). This is true also for fruits distinctly drying (Group 5) when compared with fruits in good condition (Group 2). The mature fruits (Group 3) are seen to have a slightly higher scale mortality than comparable green fruits in good condition (Group 1). Group 7 represents the data for those fruits collected at the time indicated which did not dry out. The mortality on these fruits, which were stored from 8 to 50 days, was but little higher than that on the fruits in any other group and was slightly less than that on the mature or yellow fruits. The range in mortality for the seven groups in grove A is only 2.7 per cent, whereas the ratios of live scales to total scale count show a range more than twelve times as great, or 36.7 per cent.

The mortality data for groves B and C are in harmony with the corresponding data for grove A. Grove B had been much neglected. The fruits were collected earlier than those from grove A and the storage conditions were not under such satisfactory control as for the fruits from groves A and C; also the humidity was lower in both storage media. Any or all of these factors may have exerted an influence in producing a slightly higher mortality on fruits from grove B. When the counts on fruits composed of groups most nearly equivalent from groves A and B were compared, however, the difference in mortality was found to be only 2.3 per cent (Table 2), but the percentage of live scales in the total scale count differed by 13.5.

TABLE 2.—Comparison of the ratio of living scales to living plus dying scales ( $\frac{A}{A+Dy}$ ) and the ratio of living scales to the total number of scales ( $\frac{A}{A+Dy+Dr}$ ) from combined counts on fruits consisting of groups most nearly equivalent from two different groves

Grove	Period of collection	Group No.	Fruits used	Scales				Ratios		
				A	Dy	Dr	A+Dy+Dr	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
			Number	Number	Number	Number	Number	Per cent	Per cent	Per cent
A.....	Jan. 22 to May 21, 1931.	1, 2, 3, 6	71	6, 773	198	1, 935	8, 906	97.2	2.8	76.0
B.....	Dec. 3, 1930, to Jan. 12, 1931.	8, 9, 10	70	5, 860	316	3, 207	9, 383	94.9	5.1	62.5
Total or weighted mean.....				12, 633	514	5, 142	18, 289	96.1	3.9	69.1

The mortality in the three periods, when considered separately, was found to check well with the combined mortality. The data in

Table 3 were obtained on fruits in like condition collected from one grove on two different occasions. In both cases the mortality was slightly lower for the fully developed second stage than for the two adult stages. The results for both gray adult and mature adult were somewhat different for the two sets of observations, but the average mortalities derived from the sum of the two sets of figures were practically the same for both stages.

TABLE 3.—Comparison of variability of the ratio of living scales to living plus dying scales ( $\frac{A}{A+Dy}$ ) and the ratio of living scales to the total number of scales ( $\frac{A}{A+Dy+Dr}$ ) from counts of female scales in the three later periods on fruits comparable in condition but collected at two different occasions from the same grove

Period of collection	Scales							
	Fully developed second stage				Gray adult			
	A	Dy	Dr	A+Dy+Dr	A	Dy	Dr	A+Dy+Dr
1931	Number	Number	Number	Number	Number	Number	Number	Number
Jan. 22.....	711	11	64	786	958	36	310	1,304
May 19 to 21.....	844	16	290	1,150	1,079	25	718	1,822
Total or weighted mean.....	1,555	27	354	1,936	2,037	61	1,028	3,126

Period of collection	Scales—Continued				Ratios					
	Mature adult				$\frac{A}{A+Dy}$			$\frac{A}{A+Dy+Dr}$		
	A	Dy	Dr	A+Dy+Dr	Fully developed second stage	Gray adult	Mature adult	Fully developed second stage	Gray adult	Mature adult
1931	Number	Number	Number	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Jan. 22.....	964	23	183	1,170	98.5	96.4	97.7	90.5	73.5	82.4
May 19 to 21.....	401	19	303	723	98.1	97.7	95.5	73.4	50.2	55.5
Total or weighted mean.....	1,365	42	486	1,893	98.3	97.1	97.0	80.3	65.2	72.1

In Figure 2 six frequency distributions have been plotted, in the same manner as in Figure 1, to illustrate variability of  $\frac{A}{A+Dy}$  as related to the grove from which the fruit was collected, period of collection, whether or not the fruit was stored, and, if so, at what temperature. The first four distributions (A to D, inclusive) are represented by Groups 1, 2, 7, and 8, respectively, in Table 1, and the fifth and sixth distributions (E and F) by the data for groves A and B, respectively, in Table 2. The percentages for the other groups in Table 1 are not plotted, since the number of samples was too small to bring out any significant results. However, Groups 3 and 6, and 9 and 10, are included in frequency distributions E and F, respectively.

The general trends indicated in the six frequency distributions may be stated briefly as follows: In the aggregate practically the same percentage of live scales occurs in A and B, but in A the percentage falling in the interval 97 to 98.5 per cent is more than three times that in any other interval, those in the intervals on either side of this one are nearly equal, and there is a sharply reduced percentage in the next lower interval; whereas in B the largest percentage, which is not twice that of any other interval, falls in the interval 98.5 to 100 per cent, and there is a decreasing percentage in the lower intervals. In C no interval shows a decided concentration of scales, the percentages being fairly evenly distributed in the six intervals extending from 91

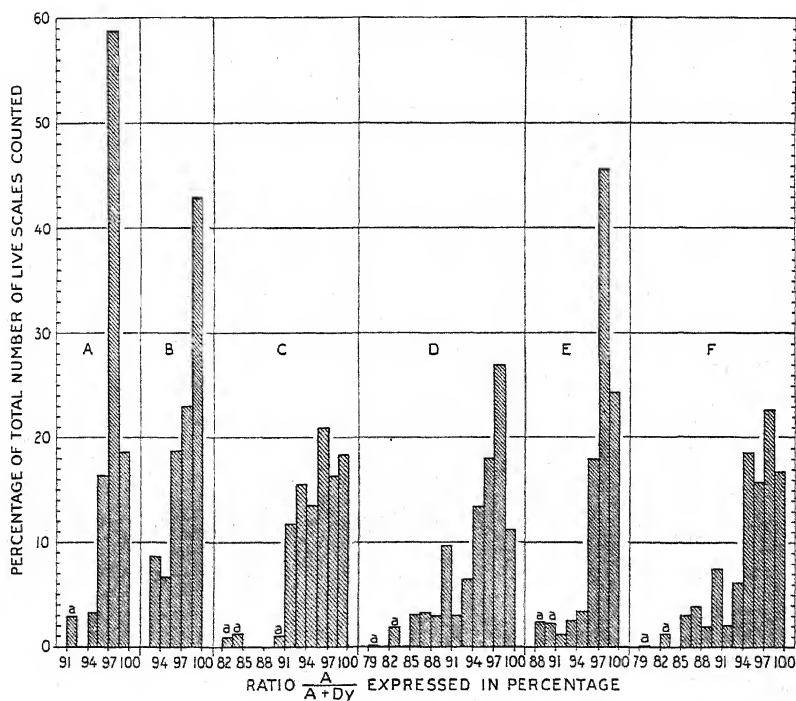


FIGURE 2.—Comparison of variability of the ratio of living scales to living plus dying scales ( $\frac{A}{A+Dy}$ ) from counts on fruits with the following factors involved: Grove from which collected, period of collection, whether stored or not, and, if so, the storage temperature. The frequency distributions are represented by the following groups in Table 1: A, Group 1; B, Group 2; C, Group 3; D, Group 4; E, Groups 1, 2, 3, and 6; F, Groups 8, 9, and 10. *a* represents the count from a single sample

to 100 per cent. In D there is some concentration of scales in the interval 97 to 98.5 per cent, but the remainder are well distributed in the next higher and in the next eight lower intervals. In E the distribution resembles that of A, but with a less marked concentration in the interval 97 to 98.5 per cent, and a correspondingly larger percentage in each of the other intervals on either side of it. In F the general range of distribution is like that of D, but more evenly distributed in the four intervals between 94 and 100 per cent. The percentage values of one to three intervals in each of the frequency distributions except one appear to be more or less aberrant; that is,

these values are larger than would be expected from the positions of their intervals, which also may be separated from the others by blank intervals. This state of affairs may be due, at least in part, to the fact that these apparent aberrant values are derived from counts on single samples.

#### METHOD OF ELIMINATING NATURAL MORTALITY IN TESTS ON FUMIGATED SPECIMENS

Thus far only the natural mortality on untreated checks has been considered. In order to use these results in experimental tests, the fumigated scales must be counted after all those killed by treatment are distinguishable from living survivors by the criteria already presented, but before they have dried out enough to be confused with those that died before treatment. When fruits were stored promptly after treatment with hydrocyanic acid gas in the controlled rooms of the lowest (13° C. or 55.4° F.) and highest temperatures (26° C. or 78.8° F.) in which approximately 78 and 72 per cent relative humidities were maintained, respectively, all dying scales in the three periods could be distinguished four days afterwards. Counts could be continued with certainty for a week in the 26° C. environment and for two weeks in the 13° C. environment.

To eliminate the natural mortality in tests on fumigated samples counted in this way, the procedure is simply to multiply the number of living plus dying scales on the treated fruits by the percentage mortality on the corresponding checks as determined by the methods given here. This gives the number of individuals that are considered to be naturally dying. On subtracting this number from the number counted as dying, the number of scales actually killed by treatment is obtained, which is then compared with the number of survivors to secure the correct percentage of mortality due to the treatment. Two examples are given in Table 4 to illustrate the elimination of natural mortality from treated samples by means of the ratios  $\frac{A}{A+Dy}$

and  $\frac{A}{A+Dy+Dr}$  of the checks. The data for the checks, besides being presented collectively (records 7 and 13) for each treatment concerned, are given individually to bring out the variability in the numbers of scales involved and in the values for the ratios. The net mortality for each set of treated samples is calculated from the collective ratio values (records 8 and 14) and also from the extreme ratio values (records 9 and 10, 15 and 16). It is observed that in both sets the collective net mortality values obtained from the ratio  $\frac{A}{A+Dy}$  are lower, and the extremes show distinctly less variability, than those obtained from the ratio  $\frac{A}{A+Dy+Dr}$ .

TABLE 4.—*Estimation of percentage of scales killed by hydrocyanic acid gas treatments using the ratio of dying scales to living plus dying scales  $\left(\frac{Dy}{A+Dy}\right)$  and the ratio of living scales to the total number of scales  $\left(\frac{A}{A+Dy+Dr}\right)$  for the elimination of natural mortality*

## EXAMPLE 1

Rec- ord No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Stor- age after collec- tion	Scales				Ratios			
						A	Dy	Dr	A+Dy+Dr	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$	
					° C.	Days	Number	Number	Number	Per cent	Per cent	Per cent	
1	Number	Check	Jan. 13, 1931		10	{	80	2	140	97.6	2.4	53.7	
2							23	0	3	100.0	0	88.5	
3							9	73	2	19	98.1	3.9	70.8
4							150	3	156	98.0	2.0	48.5	
5							94	0	36	130	100.0	0	72.3
6							96	5	53	154	95.0	5.0	62.3
7	Total or weighted mean						516	13	863	97.5	2.5	50.8	
8	{	110 per cent.	Jan. 12, 1931	Jan. 13, 1931	10	11-14	43	239	390	15.2	84.8	6.4	
10													

Rec- ord No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Mortality by writers' method			Mortality by usual method			
					A+Dy	$\frac{Dy}{A+Dy}$ of check	Killed by treatment	$\frac{A}{A+Dy+Dr}$ of check	Survivors	Killed by treatment	
					Number	Per cent	Number	Per cent	Nat- ural	Observed	
8	Number	110 per cent.	Jan. 12, 1931	Jan. 13, 1931	282	2.5	7.1	50.8	401.9	43	358.9
9					282	0	0	88.5	594.7	43	551.7
10					282	5.0	14.1	48.5	325.9	43	282.9

EXAMPLE 2

Rec- ord No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Storage tem- pera- ture	Counted after collec- tion	Scales				Ratios			
							A	Dy	Dr	A+Dy+Dr	A A+Dy	Dy A+Dy	A A+Dy+Dr	Per cent Per cent Per cent
11	Number Total or weighted mean.	Check	Dec. 6, 1930		° C. 10	Days 12	Number	Number	Number	Number	Per cent	Per cent	Per cent	Per cent
12							33	1	44	78	97.1	2.9	42.3	
13							115	2	28	145	98.3	1.7	79.3	
14							148	3	72	223	98.0	2.0	66.4	
15	Total or weighted mean.	133 per cent.	Dec. 2-5, 1930	Dec. 3-6, 1930	10	9-12	27	775	442	1,244	3.4	96.6	2.2	
16														
Rec- ord No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Mortality by writers' method				Mortality by usual method					
					A+Dy	Dy A+Dy of check	Killed by treatment	A A+Dy+Dr of check	Survivors Nat- ural	Obs- erved	Killed by treatment	Number Per cent		
14	Number	133 per cent.	Dec. 2-5, 1930	Dec. 3-6, 1930	Number	Per cent	Number	Per cent	Number	Number	Number	Per cent	Per cent	
15					802	2.0	759.0	96.50	66.4	928.0	96.73			
16					802	1.7	761.4	96.57	79.3	986.5	97.26			
17					802	2.9	751.7	96.53	42.3	926.2	94.87			

If it proves impossible to make adequate counts on natural mortality for use as given here, there is sufficiently small variation in the results obtained to justify an approximation. For female red scales from the fully developed second stage to the mature adult stage on lemons under field conditions similar to those at Whittier, Calif., a natural mortality of 4 per cent, exclusive of dried individuals, may be assumed. Though this figure is not based upon a full year's record, the work covers six months and includes the winter and spring seasons.

#### SUMMARY

In tests of hydrocyanic acid gas as a fumigant against the California red scale (*Chrysomphalus aurantii* Mask.), natural mortality as ordinarily considered varies so widely that better means of estimating it have been sought. By using lemon fruits exclusively and the three later stages in the development of the female only—that is, the fully developed second stage, the gray adult, and the mature adult—qualitative tests based on such factors as changes in body color and conformation have not given a satisfactory separation between natural mortality and mortality produced by treatment, which are mixed on treated samples, for any of these three periods. It has been found, however, that in each of the three periods natural mortality bears nearly a constant ratio to the number of living plus dying scales on untreated samples irrespective of a large number of varying factors ordinarily encountered in practice.

When counted at the proper time after treatment, practically all scales killed by treatment and those dying naturally can be distinguished, chiefly by changes in color and in viscosity of body contents. By using the relatively stable values obtained to express natural mortality, this factor can be eliminated from such counts on treated samples and a considerably higher degree of accuracy can be obtained than is possible by the old method of estimating the natural mortality from the ratio of living scales to total scale count.

This study indicates that it may be possible to determine constants for the correction of natural mortality in the California red scale, or in other insects, without separate counts for every experiment.





# THE POSSIBLE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE ABSORPTION OF POTASSIUM AND PHOSPHORUS BY WHEAT PLANTS UNDER FIELD CONDITIONS<sup>1</sup>

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## INTRODUCTION

While studying the factors affecting the absorption of mineral elements by plants, the writer showed that the hydrogen-ion concentration of the medium affects the absorption of potassium and phosphorus by wheat seedlings grown in water cultures. (4)<sup>2</sup> More phosphorus was absorbed from potassium phosphate solutions with initial pH values of 5.0 and below than from those with pH values of 6.0 and 7.0. The results for potassium were not so definite as those for phosphorus. In several cases the absorption of potassium by the wheat seedlings was higher from the nearly neutral than from the acid solutions (pH 5.0 and below); in other cases the results were not consistent.

The concentrations of potassium phosphate used in these experiments were, for reasons explained in the article referred to, relatively high, but in concentrations as low as 14 parts per million of potassium and 12 parts of phosphorus (50 parts per million of monopotassium phosphate) the general character of the results was the same. From solutions of the lower concentrations (beginning with 500 parts per million of monopotassium phosphate), the absorption of potassium by the seedlings was practically always higher when the initial hydrogen-ion concentration of the solutions had a pH value of 7.0 than when it was 4.9. In view of the fact that potassium phosphate salts are all soluble it has been concluded that this effect of hydrogen-ion concentration on the absorption of phosphorus and potassium is physiological in nature.

## EXPERIMENTAL METHODS AND DATA

It is frequently difficult to corroborate under field conditions, where a large number of known and unknown factors are dealt with, the results obtained with water cultures, where the conditions are practically controlled. Although it is true that wheat is being raised on soils with a considerable range of reaction, comparatively few chemical analyses of such crops are available, because experiment stations and other research institutions experimenting with wheat have been interested primarily in yield problems. Moreover, chemical analyses of cereals, when they are available, are largely limited to the grain, the composition of which fluctuates between narrower limits and is less subject to the influence of environment than the

<sup>1</sup> Received for publication July 22, 1932; issued April, 1933.

<sup>2</sup> Reference is made by number (*italic*) to Literature Cited, p. 452.

straw which is the vegetative part of the plant. For this reason the available data on the chemical composition of wheat are insufficient to throw light on the effect of soil reaction on the absorption and retention of the mineral elements by this plant.

In connection with an investigation on the relation of the lignin content to lodging of wheat (5), the writer was able to obtain data bearing indirectly on the effect of soil reaction on the accumulation (absorption and retention) of potassium and phosphorus by the wheat plant under field conditions. The experiment comprised two plots at the Arlington Experiment Farm, Rosslyn, Va. One served as control; the other received in the spring sodium nitrate at the rate of 600 pounds per acre. Samples for analysis were cut from the two plots at frequent intervals up to maturity. The results for the potassium and phosphorus content of the entire plant in the early stages of growth and of the stalks alone in the later stages are given in Table 1.

TABLE 1.—*Effect of applied sodium nitrate on the potassium and phosphorus content of wheat plants cut at various stages of growth*

[Percentage analyses on air-dry basis]

Date of sampling	K <sub>2</sub> O in straw from—		P <sub>2</sub> O <sub>5</sub> in straw from—	
	Control plot	Plot treated with sodium nitrate	Control plot	Plot treated with sodium nitrate
Apr. 30.....	2.5	3.63	0.73	0.52
May 7.....	2.11	3.50	.65	.51
May 14.....		3.12		.45
May 21.....	1.61	2.85	.62	.41
May 28.....	1.37	2.82	.54	.39
June 4.....	1.41	2.72	.47	.35
June 11.....	1.25	2.14	.41	.29
June 18.....	1.02	1.87	.26	.22
June 25.....	.53	.77	.22	.21

Potassium was consistently higher in all samplings from the plot treated with sodium nitrate than in the corresponding samplings from the control plot. In the case of phosphorus the reverse was true, its content being in every case higher in the straw from the control plot than in that from the fertilized plot. The results of the field experiments are therefore analogous to those of the waterculture experiment mentioned previously.

## DISCUSSION

Sodium nitrate, although it has a neutral reaction when dissolved in distilled water, is a physiologically alkaline fertilizer. The plants absorb or retain more of the nitrate anion than of the sodium cation, and as a result the reaction of the medium of growth moves toward alkalinity. It is a well-known fact that when a soil is repeatedly treated with sodium nitrate it becomes alkaline. Some of this effect undoubtedly takes place even with one application of sodium nitrate.

The distribution of plant food in the soil, especially applied plant food, is never perfectly uniform; it is concentrated at points, to which

the roots are drawn by chemotropism. Theoretically the reaction should be less acid or more alkaline at the concentration points of applied sodium nitrate than in the remainder of the soil. But this is difficult to prove in view of the fact that the reaction obtained on testing is the average reaction at the spot at which the sample was taken. Similarly, the actual concentration of the soil solution available to the plants is stronger than the apparent concentration obtained by analysis, which gives only the average concentration.

No tests for soil reaction were made in connection with this experiment, but the application of sodium nitrate was high (600 pounds per acre), and it is reasonable to assume that it affected the reaction of the soil at least at the points at which it was concentrated. The soil reaction of the section of the Arlington Experiment Farm on which the experiment was carried out has a pH value of about 6.0. A change from this reaction toward alkalinity might have affected slightly the solubility of the soil phosphates, but it could hardly be expected to have affected the solubility of the soil potassium. Furthermore, the complete similarity between these field results and those obtained in the water-culture experiment, in which the possibility of a solubility effect was entirely eliminated, would indicate that the differences in absorption of phosphorus and potassium by the wheat plants in the field experiment were due to the same physiological causes responsible for similar differences occurring in the water-culture experiment.<sup>3</sup>

The writer has suggested an explanation of the effect of hydrogen-ion concentration on the absorption of potassium and phosphorus by plants, which was based on the isoelectric relations of the ampholytes of the living cell (4). This hypothesis has been extended to include the accumulation of cations and anions in growing plants in general. It is assumed that there is a wide range in the isoelectric points of the plant ampholytes, allowing the occurrence of both electropositive and electronegative ampholytes within certain limits of hydrogen-ion concentration. This makes possible the simultaneous accumulation of cations and anions. A change in the reaction of the medium may modify, to some extent, the reaction within the plant cells and thus cause a shift of some of the electropositive ampholytes to the negative side and vice versa. This may cause an increased absorption of cations or anions, respectively, which is the explanation offered for the effect of hydrogen-ion concentration on the absorption of potassium and phosphorus by wheat plants in the parallel experiments with water cultures and under field conditions.

Briggs and Petrie (1) have subsequently suggested exactly the same hypothesis to explain the simultaneous accumulation of cations and anions in the living cell. On the other hand, Hoagland, Davis, and Hibbard (6, p. 484) oppose this hypothesis stating that—

It would be difficult to assume that individual proteins are available of such widely different isoelectric points as to permit the simultaneous formation of both cation and anion compounds at the pH values involved.

No reason is given why it is difficult to make such an assumption.

<sup>3</sup> If this explanation is correct ammonium sulphate, which shifts the soil reaction in the opposite direction from that caused by sodium nitrate, when applied under the conditions of the experiment reported in this article, would have resulted in an increase of the phosphorus content and in a decrease of the potassium content of the plant. However, the experiment was carried out with a different end in view, and the writer had no opportunity to repeat it with parallel applications of sodium nitrate and ammonium sulphate.

Our knowledge of the properties of proteins, presumably the principal ampholytes in plant tissues, is limited to isolated individual proteins which have been subjected to strenuous treatment in the process of isolation. Knowledge of the behavior of proteins in vivo is so far lacking. The same is true with regard to the available data on the hydrogen-ion concentration values of the cell sap, which are average values of sap expressed from dead or injured cells. The hydrogen-ion concentration of the sap in vivo may be entirely different. But even our limited knowledge of isolated proteins shows that proteins with quite divergent isoelectric points are present in the same plant tissue. Csonka, Murphy, and Jones (3), working with purified proteins from different sources, largely seeds, showed that the isoelectric points of the albumins are found at pH 4.0-5.0, whereas those of the prolamins are found at hydrogen-ion concentrations ranging from pH 6.0 to 6.5. Cohn, Gross, and Johnson (2) have found in potato and carrot tissue two proteins with isoelectric points at pH 4.0 and 8.0, respectively. It would seem that such differences are wide enough.<sup>4</sup>

#### SUMMARY

Wheat was grown on two field plots, one having received a relatively large application of sodium nitrate, the other serving as a control. Samples for analysis were cut at frequent intervals up to maturity.

The results for the potassium and phosphorus content of the entire plant in the early stages of growth, and of the stalks alone in the later stages, show that potassium was consistently higher in all samplings from the plot treated with sodium nitrate than in the corresponding samplings from the control plot, whereas phosphorus was higher in the samplings from the control than in those from the sodium nitrate plot.

These results are analogous to previously reported results from water cultures with controlled hydrogen-ion concentration, and are explained by a possible change in the hydrogen-ion concentration of the soil caused by the application of sodium nitrate which is a physiologically alkaline fertilizer.

A previously advanced hypothesis explaining the accumulation of cations and anions in growing plants based on the isoelectric relations of the ampholytes of the living cell is further discussed.

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<sup>4</sup> Since this article was prepared for publication there has appeared in *Soil Science* an article by Walter Thomas on The Reciprocal Effects of Nitrogen, Phosphorus and Potassium as Related to the Absorption of these Elements by Plants (7). In this otherwise full review the author, while discussing the effect of hydrogen-ion concentration of the medium of growth on the absorption of cations and anions by plants, failed to mention the hypothesis advanced by the present writer, also by Briggs and Petrie, explaining these phenomena on the basis of the isoelectric relations of the ampholytes of the living cell. Thomas seems to accept the hypothesis of Höber based on the electrostatic attraction caused by potential differences resulting from the activity of the root membranes, which varies with the reaction of the substrate. This explanation would have been adequate if the preferential accumulation of the mineral elements in the living cell were due entirely to preferential absorption. The generally accepted view, however, is that the accumulation of the mineral elements in living tissues is due to preferential retention rather than preferential absorption.

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# GERMINATION OF THE SEED OF FARM CROPS IN COLORADO AFTER STORAGE FOR VARIOUS PERIODS OF YEARS<sup>1</sup>

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## INTRODUCTION

Valuable data have been collected by various workers on the germination of seeds that have been stored a definite number of years. It is generally believed that in humid climates the seeds of farm crops lose their vitality within a few years. Under arid conditions little is known of the vitality of seeds after storage for several years. The study reported in this paper was begun with the idea of determining the viability of seeds of certain farm crops after storage for various periods.

There are very definite beliefs in various localities in regard to longevity of seeds, concerning seeds in general, and also a few particular seeds. The common belief in India (8)<sup>2</sup> is that seeds more than a year old are of no value for planting. Onion seeds are very generally considered of value only two or three years by both the seed trade and onion growers. In connection with the labeling of seeds in accordance with pure-seed laws, it is deemed advisable to retest seeds each year because germination frequently decreases materially from year to year.

## WORK OF PREVIOUS INVESTIGATORS

That seeds in sealed bottles even in India retain their viability more than one year is brought out by Sonavne (8) in a report on a 4-year storage experiment. Wheat in 1922 showed a germination of 97.6 per cent and in 1926, 98.1 per cent. Corn during the same four years deteriorated in germinating ability from 96 per cent to 86 per cent.

Eastham (4) and Sifton (7) report practically no loss in vitality of oats stored in closed boxes for 10 years. Burgerstein (1), who experimented with seeds in a closed drawer, found that barley and oats deteriorated practically none in 10 years. He found that wheat deteriorated approximately 12 per cent and rye is reduced to 2 per cent in germination. Haberlandt (6) reports that barley loses one-half its germination ability in 4 years, corn and oats one-half in 6 years, wheat practically all in 6 years, and rye all in 3 years. Welton (10) working with seeds in bottles states that in 6 years oats and wheat are reduced to a germination of approximately 20 per cent, barley to 6 per cent, and corn to 49 per cent.

Crocker and Groves (2) and Groves (5), basing their experiments on the idea that effects of temperature are similar to aging at room temperature and that the time-temperature coefficient for coagulation of proteins applies, predict the life duration of wheat as follows:

<sup>1</sup> Received for publication July 22, 1932; issued April, 1933.

<sup>2</sup> Reference is made by number (*italic*) to Literature Cited, p. 462.

Seed containing 17.5 per cent moisture held at 50° C. would survive for 17.1 days, and seeds of the same wheat at 25° would live 21.1 years. Seed containing only 9 per cent of moisture would when kept at 50° live 53 days and when held at 25° would live 37 years. Duvel (3) in an experiment to show what would happen to seeds in storage in various parts of the country concluded that in approximately 250 days there was little change if the air was dry and the temperature range normal, but that when various vegetable and flower seeds were stored in moist air there was considerable deterioration at 32° to 37°.

### EXPERIMENTAL METHOD AND RESULTS

The first tests were made on the 1920 crops. The grains were threshed, cleaned, and stored in 100-pound sacks, which were then placed in an unheated room and kept there during the entire period of the test. Samples were taken in February by mixing the grain thoroughly and drawing off a portion with a small scoop. Germination tests were made in February of each year. Crops were saved each year when grown and placed in the storage room. Only perfect seeds were used for germination, the broken and damaged seeds being discarded. The crops used were the common standard varieties of cereals shown in Table 1.

TABLE 1.—*Varieties of seed studied and years in which they were grown*

Crop	Variety	Plus mark signifies crop was grown in year indicated									
		1920	1921	1922	1923	1924	1925	1926	1927	1928	1929
Spring wheat.....	Marquis.....	-----	+	+	+	+	+	+	+	-----	+
Winter wheat.....	Kanred.....	-----	+	-----	-----	-----	-----	-----	-----	-----	-----
Spring wheat.....	Defiance.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Durum wheat.....	Kubanka.....	-----	+	-----	-----	-----	-----	-----	-----	-----	-----
	Colorado 37.....	+	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Great Dakota.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Oats.....	Swedish Victory.....	+	-----	-----	-----	-----	-----	-----	-----	-----	-----
	White Russian.....	+	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Gold Rain.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Nebraska 21.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Nepal.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Success.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Colless.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Barley.....	Coast.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Hanna.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Gold.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
	Moister.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Winter rye.....	Rosen.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Soybean.....	Wisconsin Black.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Sorghum.....	Black Amber.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Corn.....	Yellow dent.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

The average rainfall and relative humidity " $\frac{1}{2}(7A + 7P)$ "<sup>3</sup> in percentage of saturation is given in Table 2. These data were taken from Bulletin 340 (9, *Tables 17 and 21*)<sup>4</sup> of the Colorado Agricultural Experiment Station.

<sup>3</sup> To obtain the relative humidity, readings were made twice daily at 7 a. m. and 7 p. m., and the average of the two readings taken as the mean (9, p. 11, and Table 17).

<sup>4</sup> Data for 1928, 1929, and 1930 were obtained directly from Mr. Trimble.



TABLE 2.—*Rainfall and relative humidity during period covered by experiments*

Year	Average annual precipitation	Average annual relative humidity	Year	Average annual precipitation	Average annual relative humidity	Year	Average annual precipitation	Average annual relative humidity
	<i>Inches</i>	<i>Per cent</i>		<i>Inches</i>	<i>Per cent</i>		<i>Inches</i>	<i>Per cent</i>
1920.....	11.65	70.2	1924.....	10.64	64.8	1928.....	13.54	65.3
1921.....	14.83	66.2	1925.....	14.50	64.3	1929.....	13.73	66.8
1922.....	9.98	67.6	1926.....	13.56	66.4	1930.....	15.17	64.8
1923.....	27.57	71.6	1927.....	15.77	67.2			

There appears to be no connection between the average annual relative humidity and the original germination percentage. Humidities for all years were low. The actual percentage of moisture in the seed samples was determined for the 1929 crop and was found to range between 9.5 and 11.4 per cent. No tests were made on the other crops.

The germination tests made the year the seeds were harvested ranged from 93 to 98.5 per cent for Marquis, 84 to 90 per cent for Kubanka, 93 to 95 per cent for Kanred wheat. (Table 3.) Barley and rye showed a greater variation from year to year and for different varieties, while the percentage of germination of oats did not differ greatly from that of wheat.

TABLE 3.—*Germination percentages for various seeds harvested in different years*

Crop	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929
Wheat:										
Marquis.....		98.0	97.5	93.0	93.5	97.5	95.5	98.5		97.0
Kubanka.....		88.0	90.0							84.0
Kanred.....		95.0			94.0					93.0
Barley:										
Nepal.....	87.0	95.5	95.0	73.0	69.0	84.0				
Colless.....			99.5	98.5	97.0	97.0	94.0	97.0	97.0	99.5
Coast.....	97.0	94.0		98.5	92.0	96.5				
Moister.....						98.5	96.0	97.0		
Oats:										
Colorado 37.....	98.0		99.0	99.0	98.0	97.0	97.0	95.0	98.5	98.0
Nebraska 21.....			97.0	97.5	95.5	94.0	98.0		92.0	
Miscellaneous crops:										
Rye.....			98.0	83.5	84.0					
Soybeans.....				93.5						
Sorghum.....								92.0		
Corn.....				85.0	98.0					

The general trend seems to indicate that oats, covered barley, Marquis and Kanred wheat have a high percentage of germination the first year, while Kubanka wheat, Nepal barley (naked), and rye show a comparatively low germination percentage the first year.

A study of the germination of the various seeds as a percentage of the original germination shows that when all varieties of the same crop are averaged there is a slow decline in the germination percentage for the first 10-year period. Table 4 gives the average percentage of germination for all seeds studied, as well as the average for each variety, and the number of years in the test period.

## WHEAT VARIETIES

The table gives the average germination for all wheat seed as well as the average for each variety. Three varieties of *Triticum vulgare* and one variety of *T. durum* were tested. The commercial classes are represented by three varieties. Marquis is a hard red spring wheat. The germination percentage of this variety falls off 8.7 per cent in 10 years. Kanred, a hard red winter wheat, shows a similar drop. Defiance, a soft white wheat, shows the same general trend but drops off less than Marquis.

TABLE 4.—Germination percentages of wheat, barley, oats, and miscellaneous seeds grown in Colorado when the original test is valued at 100 per cent and the other tests are calculated as percentage of the first year's test

Crop and variety of seed	Percentage germination and number of crops tested in indicated number of years after harvesting									
	1	2	3	4	5	6	7	8	9	10
Wheat:										
Marquis—										
Germination.....per cent..	100	99.8	98.1	97.5	96.7	95.1	95.9	93.1	93.4	91.3
Crops tested.....number..	9	8	7	7	6	5	4	3	2	1
Defiance—										
Germination.....per cent..	100	98.4	99.3	98.2	96.7	97.9	96.2	96.4	94.3	-----
Crops tested.....number..	4	4	4	4	3	2	2	1	1	-----
Kanred—										
Germination.....per cent..	100	102.5	98.2	100.5	97.8	96.9	94.5	93.7	90.1	98.4
Crops tested.....number..	3	3	2	2	2	2	2	1	1	1
Kubanka—										
Germination.....per cent..	100	100.7	97.8	97.5	95.0	98.7	94.2	92.5	84.9	89.8
Crops tested.....number..	3	3	2	2	2	2	2	2	2	1
Average germination of all wheats										
.....per cent..	100	100	98.4	98.1	96.6	96.6	95.3	93.4	90.2	93.2
Total number of crops tested.....	19	18	15	15	13	11	10	7	6	3
Standard error.....	±0.93	±0.66	±0.80	±1.15	±1.19	±1.44	±1.15	±1.15	±2.27	-----
Barley:										
Nepal—										
Germination.....per cent..	100	99.3	89.8	86.2	88.3	85.9	92.3	83.2	81.3	75.8
Crops tested.....number..	6	6	4	6	6	6	5	4	3	2
Success—										
Germination.....per cent..	100	97.8	95.3	97.0	98.5	94.1	96.4	94.9	89.8	89.1
Crops tested.....number..	3	3	1	2	2	3	3	3	3	2
Colless—										
Germination.....per cent..	100	98.5	97.6	98.7	96.8	95.2	93.5	96.0	93.9	-----
Crops tested.....number..	8	8	7	6	5	4	3	2	1	-----
Coast—										
Germination.....per cent..	100	100.5	100	99.6	98.6	98.8	98.3	96.9	98.5	101.4
Crops tested.....number..	5	5	5	5	5	5	4	3	2	2
Hanna—										
Germination.....per cent..	100	97.3	104.3	99.3	100.4	94.9	90.2	90.8	90.7	86.7
Crops tested.....number..	2	1	2	2	2	2	2	2	2	1
Gold—										
Germination.....per cent..	100	97.4	99.5	98.4	93.7	87.4	81.0	79.1	78.0	68.1
Crops tested.....number..	1	1	1	1	1	1	1	1	1	1
Moister—										
Germination.....per cent..	100	99.8	99.3	99.6	94.4	95.9	-----	-----	-----	-----
Crops tested.....number..	3	3	3	3	2	1	-----	-----	-----	-----
Average germination of all barley										
.....per cent..	100	99.0	97.6	95.9	95.1	93.0	93.7	90.7	88.6	85.9
Total number of barley crops tested.....	28	27	23	25	23	22	18	15	12	8
Standard error.....	±1.40	±0.63	±1.04	±1.56	±1.48	±1.66	±2.95	±1.80	±2.11	-----
Average germination of naked barley.....per cent..	100	99.3	89.8	86.2	88.3	85.9	92.3	83.2	81.3	75.8
Total number of crops of naked barley tested.....	6	6	4	6	6	6	5	4	3	2
Average germination of 6-row hooded covered barley.....per cent..	100	98.3	97.3	98.2	97.2	94.7	94.9	95.3	90.8	89.1
Total number of crops of 6-row hooded covered barley tested.....	11	11	8	8	7	7	6	5	4	2
Average germination of 6-row covered awned barley.....per cent..	100	100.2	99.8	99.6	97.4	98.3	98.3	96.9	98.5	101.4
Total number of crops of 6-row covered awned barley tested.....	8	8	8	8	7	6	4	3	2	2
Average germination of 2-row covered awned barley.....per cent..	100	97.4	102.6	99.0	98.1	92.4	90.5	86.9	77.4	79.8
Total number of crops of 2-row covered awned barley tested.....	3	2	3	3	3	3	3	3	2	2

TABLE 4.—*Germination percentage of wheat, barley, oats, and miscellaneous seeds grown in Colorado when the original test is valued at 100 per cent and the other tests are calculated as percentage of the first year's test—Continued.*

Crop and variety of seed	Percentage germination and number of crops tested in indicated number of years after harvesting									
	1	2	3	4	5	6	7	8	9	10
Oats:										
Colorado 37—										
Germination.....per cent..	100	101.4	100.8	97.8	95.5	95.3	95.3	94.6	95.0	91.9
Crops tested.....number..	9	8	8	7	6	5	4	3	2	1
Great Dakota—										
Germination.....per cent..	100	97.8	97.8	95.7	96.5	94.8	93.0	95.5	89.4	-----
Crops tested.....number..	2	2	2	2	2	2	2	2	2	-----
Swedish Victory—										
Germination.....per cent..	100	95.0	99.3	97.8	93.5	92.7	94.5	92.7	91.0	81.8
Crops tested.....number..	2	1	2	2	2	2	2	2	2	1
White Russian a—										
Germination.....per cent..	-----	98.5	-----	97.0	98.0	95.0	93.5	94.5	97.5	88.0
Crops tested.....number..	1	1	-----	1	1	1	1	1	1	1
Gold Rain—										
Germination.....per cent..	100	99.5	99.9	97.1	98.5	91.6	-----	-----	-----	-----
Crops tested.....number..	2	2	2	2	2	1	-----	-----	-----	-----
Nebraska 21—										
Germination.....per cent..	100	100.6	97.7	97.3	95.7	95.7	98.5	88.1	92.8	-----
Crops tested.....number..	7	6	6	5	3	3	2	1	1	-----
Average germination of all oats										
.....per cent..	100	100.1	99.4	97.3	95.9	94.7	95.1	93.7	92.6	87.2
Crops tested.....number..	23	20	20	19	16	14	11	9	8	3
Standard error.....	±0.44	±0.97	±0.91	±0.68	±0.72	±0.68	±0.87	±0.94	±2.65	-----
Average germination of midseason oats.....per cent..	100	100.2	100.0	97.4	95.3	94.6	94.5	94.3	92.3	86.9
Total number of crops tested...	13	11	12	11	10	9	8	7	5	2
Rosen rye:										
Germination.....per cent..	100	98.1	98.4	90.6	90.1	89.6	79.8	79.1	52.0	-----
Crops tested.....number..	4	3	3	3	3	3	3	3	1	-----
Wisconsin black soybeans:										
Germination.....per cent..	100	101.1	93.0	86.6	90.1	83.3	61.2	48.1	-----	-----
Crops tested.....number..	2	2	2	2	2	2	2	1	-----	-----
Black Amber sorghum:										
Germination.....per cent..	100	117.0	119.0	115.8	104.4	97.9	-----	-----	-----	-----
Crop tested.....number..	3	3	3	3	2	1	-----	-----	-----	-----
Corn:										
Germination.....per cent..	92	90	97	97	87	88.4	86.9	80.2	79	-----
Crops tested.....number..	2	1	2	2	3	5	6	5	2	-----

a Actual percentages, because of lack of test the first year.

Kubanka, a durum wheat, drops off slightly for eight years and then makes a quick drop. However, further tests may smooth out the discrepancy.

The average of all wheats tested shows a gradual drop for a 9-year period. There is a slight rise in the tenth year, but as only three years are represented in this test, less reliance can be placed on it. The standard errors were worked for all years up to the ninth year and show uniformity, with the exception of the ninth year which shows a wider variation, due, possibly, to the low number of years in the test. Wheat seed stored in a dry unheated room lose about 7 per cent of their germination in a 10-year period under Colorado conditions.

The actual germination of all wheat varieties tested is given in Table 5. This table represents the types of data obtained with the barley and oats studied in the germination experiment.

TABLE 5.—Germination percentage of different wheat varieties grown at Fort Collins, Colo., 1921-1930

Variety	Year grown	Percentage of germination in indicated number of years after harvesting									
		1	2	3	4	5	6	7	8	9	10
Marquis.....	1921	98.0	97.0	96.0	92.5	93.5	92.5	94.5	88.5	94.0	89.5
	1922	97.5	97.5	99.0	98.5	97.0	97.5	93.0	94.0	88.5	-----
	1923	93.0	94.5	83.0	81.5	86.5	79.0	84.0	86.0	-----	-----
	1924	93.5	96.5	96.5	93.5	95.0	92.5	95.0	-----	-----	-----
	1925	97.5	93.5	94.5	87.5	88.5	95.0	-----	-----	-----	-----
	1926	95.5	96.5	93.0	95.0	95.5	-----	-----	-----	-----	-----
	1927	98.5	96.5	99.0	98.5	-----	-----	-----	-----	-----	-----
	1929	97.0	96.5	-----	-----	-----	-----	-----	-----	-----	-----
	1930	97.0	-----	-----	-----	-----	-----	-----	-----	-----	-----
	1921	95.0	97.0	94.0	95.5	93.5	92.5	88.0	89.0	86.0	93.5
Kanred.....	1924	94.0	95.0	91.5	94.5	91.0	90.5	90.5	-----	-----	-----
	1929	93.0	97.0	-----	-----	-----	-----	-----	-----	-----	-----
	1922	96.0	95.0	96.0	94.0	95.0	94.0	95.0	92.5	90.5	-----
Defiance.....	1924	89.0	91.0	88.5	91.5	89.5	87.0	83.0	-----	-----	-----
	1926	90.5	86.0	90.5	85.5	82.0	-----	-----	-----	-----	-----
	1927	98.5	96.0	96.5	96.0	-----	-----	-----	-----	-----	-----
	1921	88.0	92.5	86.5	89.5	88.5	91.5	86.0	84.5	79.0	79.0
Kubanka.....	1922	90.0	86.5	87.5	84.0	80.5	84.0	81.5	80.0	72.0	-----
	1929	84.0	84.5	-----	-----	-----	-----	-----	-----	-----	-----

## BARLEY VARIETIES

The results of the barley varieties tested are also shown in Table 4. The 6-row hooded covered barleys decreased in germination about 10 per cent in 10 years. However, Coast showed a drop of only about 3 per cent. Nepal, a hull-less barley, dropped off about 20 to 25 per cent, the decrease starting about the third year. Two varieties of 2-row barley were tested, and they showed a more marked drop than did the 6-row barleys.

When the average of all barleys grown is considered, there is a gradual drop in percentage germination with indications of a greater drop after the ninth year. The total drop equals 14.1 per cent in 10 years, which is slightly greater than that of all wheat varieties.

If only 6-row hooded covered barleys are considered, there is slightly less falling off than with wheat. There seems to be a greater tendency for naked and 2-row barleys to lose vitality than for 6-row covered awned varieties. The standard error is slightly higher than that for wheat. However, it still shows a significant difference between the first and ninth year tests.

## OAT VARIETIES

Both midseason and early oats were used in this test. Only varieties of *Avena sativa* and *A. sativa orientalis* were used in the experiment. Colorado No. 37, a high yielding oat of the midseason type, decreased in germination 8.1 per cent in 10 years. (Table 4.) Great Dakota showed a similar tendency. Swedish Victory decreased to 93.5 per cent in the fifth year and remained fairly uniform until the eighth year. A slight drop was noticed in the ninth year and a marked drop in the tenth year. Only two lots of this oat were used in the test, and the tenth year is represented by only one lot. White Russian, a side oat, decreased slightly until the eighth year, then increased in the ninth and decreased in the tenth year, dropping 12 per cent.

Nebraska 21, an early oat, shows a similar trend to the midseason oats, decreasing slightly each year, with slight variations, possibly due to sampling error.

When the average of all midseason oats tested is considered, there is a gradual decrease in germination for nine years, with a marked drop in the tenth year, when only two lots were tested. A very similar condition is found when all oats tested are considered.

#### OTHER SEEDS

Less complete data were obtained for other seeds. Rosen, a winter rye, decreased noticeably after the third year. Three lots of this variety were used in the test and by the seventh year, a drop of 20.2 per cent had been obtained. More data are necessary before final conclusions for the ninth and tenth years can be drawn.

Only one variety of soybeans was tested. Wisconsin Black, an early maturing bush type of bean, was used. This variety decreased in germination percentage after the fifth year and continued to drop rapidly after that period. Varying percentages of hard seeds were found in the soybeans tested. The number, however, fell off gradually until the fourth year, when all viable seeds germinated.

Black Amber, a forage sorghum adapted to northern Colorado, had lost very little of its viability after being stored for six years.

Several varieties of yellow dent corn were used. The test for corn is the most incomplete of the series. However, the data were included because of the increase of acreage of this crop in the past 10 years. Corn held its germination percentage well for a 4-year period. After this stage it decreased to a marked extent and again remained uniform until the seventh year, after which it again commenced to drop, losing 21 per cent of its germination by the ninth year.

The data for the last four kinds of seed are less conclusive than those for wheat, barley, and oats. However, they indicate that other farm seeds, such as winter rye, soybeans, sorghum, and corn stored in dry rooms may be used after five years with only a slight increase in the rate of seeding in order to obtain a good stand.

#### SUMMARY

Germination tests were made on the seed of various cereal crops adapted to Colorado conditions after being stored for varying periods of years in a dry room.

The germination percentage of wheat decreased about 7 per cent in 10 years.

The germination percentage of barley decreased about 14 per cent in 10 years. Two-row and naked barley decreased more than hulled 6-row barley.

The germination percentage of both midseason and early oats decreased about 13 per cent in 10 years.

Rosen rye and Wisconsin Black soybeans decreased 10 per cent in germination in 5 years. After the fifth year the germination percentage decreased rapidly.

Black Amber sorghum germinated well for six years, decreasing 2 per cent in this time.

Yellow dent corn germinated well for the first four years but decreased 13 per cent in the fifth year. There was a decrease of 20 per cent after eight years of storage.

Small-grain crops stored in a dry, unheated room, under the climatic conditions at Fort Collins, have still a high percentage of viable seed when 10 years old.

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# THE RELATIVE DIGESTIBILITY BY RATS OF THE MILK OF FIVE BREEDS OF DAIRY CATTLE<sup>1</sup>

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## INTRODUCTION

More than 40 per cent of the milk produced in the United States is consumed as fluid milk, while more than 30 per cent is used in the manufacture of butter. About 3 per cent of the milk is used in calf feeding. Much of the skim milk and buttermilk resulting from butter making is fed to livestock. The thoroughness with which the components of whole milk and also those of creamery by-products fed to livestock are digested, is a matter of economic importance.

Because of differences in the chemical composition and physical characteristics of the milk of the breeds of dairy cattle, a question naturally arises regarding the relative digestibility of the milk of these breeds. The greatest difference in chemical composition lies in the percentage fat content, but differences are found also in the percentages of protein, lactose, and ash (21).<sup>4</sup> Differences exist in the size of the fat globules and the rapidity with which they rise (6, p. 143).

Much interest in the relative digestibility of the milk of the breeds of dairy cattle has been aroused through the observations of digestive disturbances in artificially fed infants receiving a milk diet. Some of these disturbances seem to follow the ingestion of milk high in fat content, indicating a limited capacity of certain individuals for the digestion of fat. The work of Hill (9) has shown that the protein in the milk of some cows forms a "soft curd" while that in the milk of other cows forms a "hard curd." The soft-curd milk has been found of value for infants suffering from digestive troubles. Several experiments upon the completeness of digestibility of milk fat by adults show slight differences in the digestive coefficients. It seems evident, therefore, that several factors may be involved in the digestion of milk, particularly in the case of infants. It is clear that a thorough study of the digestibility of milk by infants would necessitate employing them as subjects.

The only phase of digestibility studied in the experiments reported in this paper is the completeness of digestibility of the components of milk. The investigation was prompted by the fact that the authors know of no animal experiments which would afford direct comparisons of the completeness of digestibility of the milk of the various dairy-cattle breeds. It is not assumed that the experiments have any bearing upon digestive troubles either of infants or of adults, or upon the time required for gastric digestion, or upon any phase of the

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<sup>4</sup> Reference is made by number (*italic*) to Literature Cited, p. 471.

digestive process other than completeness of digestibility. In fact, since the digestibility of milk by infants appears to involve so many factors, the authors disclaim any attempt to apply the results of these experiments to the field of infant nutrition.

The use of albino rats naturally raises a question concerning the application of the results to other species. While the results of nutrition studies conducted with rats may not apply in toto to other species, it is believed that in experiments such as the present, in which comparative results were sought, the findings may be applied to a reasonable extent to the human adult and to animals of other species which normally consume milk, such as young calves and pigs. For example, should experiments with rats show pronounced differences in the digestibility of the milk of two breeds of dairy cattle, it is only reasonable to assume that about the same relative differences in digestibility would be shown by other species which normally consume milk, although the actual digestion coefficients secured with various species might differ considerably.

### REVIEW OF THE LITERATURE

On account of the fact that the greatest difference in the chemical composition of the milk of the dairy-cattle breeds lies in the percentage fat content, the study of the digestibility of the fat was a point of special interest. The review of the literature, therefore, has been limited to experiments in which the completeness of digestibility of milk fat was determined. This is presented in condensed form in Table 1.

TABLE 1.—Condensed review of the literature

Investigator	Subject	Food	Coefficient of apparent digestibility		
			Fat	Protein	Dry substance
Rubner (22).....	Human.....	Milk only.....	95.4	93.0	91.6
			94.4	92.3	90.6
			92.9	88.0	89.8
Do.....	do.....	Butter <sup>a</sup> .....	96.3		
			97.3		
Rubner (23).....	do.....	do <sup>a</sup> .....	91.5		
Mayer (18).....	do.....	do <sup>a</sup> .....	98.3		
Atwater (2).....	do.....	do <sup>a</sup> .....	97.1		
Do (3).....	do.....	Milk.....	91.0		
Do (4).....	do.....	Butter <sup>a</sup> .....	97.0	88.1	
			96.7		
Hultgren and Landergren (16).....	do.....	do <sup>a</sup> .....	97.3		
			94.6		
Bryant (5).....	do.....	Milk only.....	95.7	93.8	
Lührig (15).....	do.....	Butter <sup>a</sup> .....	95.7		
Do (16).....	do.....	do <sup>a</sup> .....	96.9		
			97.2		
Kienzl (15).....	do.....	do <sup>a</sup> .....	96.7		
Wibbens and Huizenga (24).....	do.....	do <sup>a</sup> .....	97.3		
			96.5		
Doane and Price (7).....	Dairy calves.....	Milk only.....	96.8	94.8	
Do (8).....	do.....	do.....	96.1	92.6	
Von Gerlach (10).....	do.....	do.....	97.2	93.5	
Langworthy and Holmes (12).....	Human.....	Butter <sup>a</sup> .....	97.0		
Do (13).....	do.....	do <sup>a</sup> .....	93.9		
Do (14).....	do.....	Cream <sup>a</sup> .....	92.7		
Lundgren, Ferander, and Putkonen (17).....	do.....	Butter <sup>a</sup> .....	94.6		
		do <sup>a</sup> .....	94.8		
Holmes (10).....	do.....	do <sup>a</sup> .....	97.0		
			96.1		
Hughes and Cave (11).....	Dairy calves.....	Milk <sup>b</sup> .....	98.5	95.7	
			99.0	94.8	

<sup>a</sup> As part of a simple mixed diet.

<sup>b</sup> Partially skimmed.



## EXPERIMENTAL METHODS

It is assumed that the differences between the amounts of protein, fat, sugar, and total solids in the milk consumed and the amounts of these excreted in the feces represent the amounts digested. It is believed that determinations of digestibility made in this way are accurate even though products of endogenous origin are present in the feces. The amount of these products is undoubtedly closely related to the amount and character of the food ingested and may be considered one of the necessary costs of digestion. Because the method of calculation used in these studies makes no allowance for the presence of the endogenous substances in the feces, the term "apparent digestibility" is used.

The dairy breeds whose milk was compared were Jersey, Guernsey, Holstein-Friesian, Ayrshire, and Brown Swiss. The trials were conducted with albino rats. In all the trials except those with Brown Swiss milk, healthy rats from the department's stock colony were used. In order to keep them in good physical condition, they were fed the mixture used for the stock colony for a few days following each digestion trial. The studies with Brown Swiss milk were conducted in connection with a growth experiment in which the animals became anemic. These received fresh whole milk only from time of weaning, with no changes whatever in the manner of feeding.

The animals were confined individually in glass cages below which pans lined with heavy filter paper were placed to receive the excreta. The rods in the bottoms of these cages were spaced far enough apart so that the feces dropped through very readily and rarely lodged in the cage. In the digestion trials for protein, clean filter papers were placed in the pans daily; in trials for the other components, they were renewed on alternate days.

The digestion trials were 10 days in length. Each was preceded by a preliminary period of 3 to 5 days, during which the particular milk to be tested was fed. Milk was the only food given during the digestion trials. The amounts fed were adjusted during the preliminary periods to levels slightly below the maximum amounts which the animals would consume. Efforts were made to keep the daily intake for each animal constant throughout a 10-day trial. In most of the trials, paired feeding was practiced so that the animals of a pair consumed different kinds of milk in the same amounts. In these trials, two animals of the same sex and of approximately the same weight formed a pair.

The milk was secured from the university dairy barns daily. The containers were washed thoroughly each day with washing powder and then rinsed with a concentrated sulphuric acid-sodium dichromate cleaning solution. The milk was kept in a refrigerator and was never more than 24 to 28 hours old when fed. Even though unheated, it was always in excellent condition. Feedings were given twice daily. The containers used were glass sponge dishes, and after each feeding these dishes were carefully washed, rinsed with distilled water, and drained on a drying rack constructed of glass rods. The careful sanitary methods used are believed to have been responsible for the fact that practically no cases of milk souring occurred. The feed records, therefore, are uncomplicated by irregular or lowered intake of milk on account of its condition.

The Brown Swiss milk, as secured daily, consisted of the mid-portion of a single milking of an individual cow. The milk of three different Brown Swiss cows was used, but in no case was the milk of more than one of these cows used in any one digestion trial. About equal numbers of coefficients were secured with the milk of the three cows. The milk of the other breeds was taken daily from the mixed milk of a single complete milking of each of four cows. The milk was taken at a time when the cows were neither near the beginning nor the close of their lactation periods.

It is recognized that the number of cows employed is small. This procedure is believed to be justified in view of the fact that this study had for its chief object the discovery of any differences which may exist in the digestibility of the milk of the dairy-cattle breeds. It has been shown (21, Table 6) that the variations in the composition of the milk of individual cows within a breed are far greater than the differences between the mean values of the milk components of the various breeds. The use of samples of the mixed milk of a large number of cows of a breed for digestibility studies, therefore, would be likely to minimize and obscure any differences between breeds, while the use of samples of milk from individual cows or from small groups of cows would be more likely to demonstrate differences, should any exist.

Samples were taken from each lot of milk before feeding. Small amounts of formalin were added and the samples were kept in a refrigerator until analyzed. Analyses were made of composite samples.

The feces were removed from the collection pans daily. In the digestion trials for the determination of protein, the feces were transferred directly to stoppered Kjeldahl flasks containing 50 c c chemically pure sulphuric acid. When it was evident that some of the feces had been contaminated by urine, they were transferred, before being placed in the Kjeldahl flasks, to a sheet of clean filter paper laid over hardware cloth and washed by a stream of hot, slightly acidified, nitrogen-free water from a wash bottle. This procedure was changed in the last two trials. Trays made of hardware cloth having eight meshes to the inch were placed below the cages on top of the collection pans and the feces were transferred from the trays directly to the Kjeldahl flasks with no washing in any case. In the second of these last two trials, the rats were carefully clipped to prevent hair from being ingested or falling into the collection trays and adhering to the feces.

In the trials for the determination of fat, total solids, and sugar, the feces were transferred daily to paraffined paper cups, in which they were dried for two to three hours at a temperature of 60° to 70° C. They were then stored in glass jars in a refrigerator until analyzed.

The methods of analysis prescribed by the Association of Official Agricultural Chemists (1) were followed. Qualitative tests only for sugar were made, both Benedict's and Fehling's methods being used.

#### PRESENTATION OF RESULTS

A summary of the digestion coefficients secured is given in Tables 2, 3, and 4. Most of the detailed data secured with Brown Swiss milk and a few of those obtained with Holstein milk have been presented in previous papers (19, 20).

TABLE 2.—Comparative apparent digestibility of the fat of the milk of dairy cattle <sup>a</sup>

	Summary of coefficients of apparent digestibility of milk fat				
	Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
	99.1	99.6	98.6	98.9	97.1
	98.6	99.7	98.9	98.5	98.8
	98.4	99.6	97.4	98.6	98.5
	98.1	99.7	98.1	98.1	98.6
	99.3	98.8	99.2	98.7	98.4
	97.8	99.5	98.7	98.0	98.2
	98.9	99.4	98.4	98.7	98.9
	98.2	99.4	99.2	98.9	98.7
	98.7	99.4	98.7	98.4	98.7
	98.6	99.4		99.0	98.5
		99.3		95.5	
		99.6		96.5	
		99.3		98.8	
		99.5		98.5	
		99.7		98.2	
		99.5		98.3	
		99.6		97.6	
		99.2		97.9	
		99.0		99.0	
		99.5			
		98.0			
		99.4			
		98.8			
		98.8			
		98.3			
		97.9			
		98.2			
		98.1			
		98.4			
		98.1			
		98.4			
		98.9			
		98.7			
		98.4			
Mean.....	98.6 ± 0.09	99.0 ± 0.07	98.6 ± 0.11	98.2 ± 0.13	98.4 ± 0.10
Standard deviation.....	.44 ± .07	.57 ± .05	.49 ± .08	.84 ± .09	.49 ± .07
Coefficient of variability.....	.44 ± .07	.58 ± .05	.50 ± .08	.86 ± .09	.49 ± .07

<sup>a</sup> Number of determinations. Ayrshire, 10; Brown Swiss, 34; Guernsey, 9; Holstein-Friesian, 20; Jersey, 10.

The fat of the milk of dairy cattle was found to be highly digestible. (Table 2.) When it is considered that extraction of feces with warm ether for removal of the fat almost certainly removes ether-soluble substances other than food fat residues, it seems reasonable to assume that there was very little if any undigested milk fat in the extracts. The conclusion naturally follows that the fat of fresh whole milk fed in these experiments was nearly all, if not entirely all, digested.

Practically no differences in the apparent digestibility of the milk fat of the five breeds were found. (Table 2.) The means of the coefficients for Ayrshire, Brown Swiss, Guernsey, and Jersey milk are practically the same. Moreover, the standard deviations and coefficients of variability are very similar. The mean of the coefficients for the fat of Holstein milk is slightly below that of the other breeds, caused in part by two unexplainably low coefficients, namely, 95.5 and 96.5. Such variations in results are likely to occur in biological investigations of this sort and no special significance should be attached to them. In fact, it is rarely possible to secure results of 10-day digestion trials which agree as closely as the 83 values shown in Table 2. With the exception of these two low coefficients, all coefficients for the five breeds are above 97, and only six others are below 98. If the two lowest coefficients for Holstein milk (95.5 and 96.5) be omitted, then the mean of the coefficients is  $98.5 \pm 0.06$ , a value which is nearly the same as the values for the other breeds.

TABLE 3.—Comparative apparent digestibility of the total protein of the milk of dairy cattle <sup>a</sup>

	Summary of coefficients of apparent digestibility of milk protein				
	Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
<i>a</i>		<i>d</i>			
87.5		95.8	91.0	91.6	88.2
90.6		95.7	88.8	92.6	92.9
91.0		93.4	92.2	92.3	91.7
90.5		92.8	87.3	92.4	89.2
91.2			93.1	94.1	90.0
88.7		93.7	88.4	94.7	88.4
88.1		94.1	88.7	93.0	90.4
89.2		94.1	88.9	93.1	87.2
90.6		96.1	88.5	88.4	91.6
95.3		94.3		88.2	87.0
<i>b</i>		94.2		89.7	
91.9		94.8		88.4	
89.8				89.9	
90.3		<i>f</i>		92.6	
89.2		94.5		89.4	
91.7		96.1		85.8	
88.0		93.0		91.4	
89.1		92.4		90.6	
89.6		96.5			
91.4		94.7		<i>a</i>	
93.4		94.4		89.3	
<i>c</i>		<i>g</i>		86.9	
89.7		88.9		91.1	
89.6		91.9		87.9	
90.2		90.7		90.9	
88.0		90.2		86.9	
89.5		89.9		91.7	
88.4		92.7		87.2	
89.2		92.3		86.9	
90.3		92.3		89.9	
87.1		89.6		<i>b</i>	
91.1		91.6		89.5	
		91.9		88.1	
		91.3		90.2	
		89.8		87.6	
		93.6		89.1	
		91.5		85.1	
		91.0		92.1	
		93.0		86.8	
		93.3		89.8	
		90.5		88.3	
		91.6		<i>c</i>	
		90.7		90.6	
		93.5		90.5	
		92.4		89.1	
		92.4		87.9	
		89.0		91.7	
		90.5		87.2	
		91.2		92.3	
		91.1		87.0	
		91.2		90.3	
		91.6		91.3	
		91.2			
		92.6			
		93.4			
		91.7			
		91.5			
		92.5			
		93.4			
		92.9			
		92.1			
		90.9			
		92.3			
		92.7			
		92.4			
		<i>h</i>			
		90.9			
		92.2			
		91.1			
		90.2			
		91.3			
		90.9			
		92.9			
		91.0			
		92.4			
Mean.....	90.0±0.21	92.3±0.14	89.7±0.36	89.8±0.22	89.7±0.41
Standard deviation.....	1.7±.15	1.7±.10	1.6±.26	2.3±.15	1.9±.29
Coefficient of variability.....	1.9±.16	1.8±.10	1.8±.29	2.5±.17	2.1±.32

<sup>a</sup> Number of determinations: Ayrshire, 30; Brown Swiss, 70; Guernsey, 9; Holstein-Friesian, 48; Jersey, 10.

These coefficients for fat are higher, upon the whole, than those reported in the literature. It is likely that this difference may be explained by differences in the methods of experimental procedure employed. In most of the investigations cited, the experimental periods were of one to three days' duration. In many cases the digestive tract may not have been cleared of food residues from diets taken previous to the experimental diet, but instead suitable substances were administered for marking the feces. Probably a more important difference than either of those just mentioned is the fact that in many of the experiments cited, the milk fat was taken as part of a mixed diet. In such cases it is likely that more nonfat substances are extracted from feces by ether than is the case when the diet consists of milk only. In a number of cases this alone might account for the fact that the coefficients for fat recorded in the literature were lower than those reported in this paper.

The apparent digestibility of the total protein was found to be lower than that of the other components studied. (Table 3.) There is some variation in the data, but, on the whole, the results are remarkably uniform. Of the 167 coefficients shown in Table 3, each of which was derived by means of a 10-day digestion trial, only 6 fall below 87 and only 6 exceed 95. The mean of the 167 determinations is  $90.9 \pm 0.12$ .

There are small differences in the data obtained for the various breeds. The means of the coefficients of the Ayrshire, Guernsey, Holstein, and Jersey breeds are nearly identical. It would be extremely difficult to obtain results more nearly alike than these with two groups of animals, even if all animals were fed the same kind of milk.

A possible explanation of the fact that the coefficients of apparent digestibility for the protein of Brown Swiss milk are higher than those for the milk of the other breeds is that the values in the first column under Brown Swiss (Table 3) were secured under different conditions from the others. These results were obtained in growth experiments with young rats. The first four coefficients (*d*) were secured with animals weighing 107 to 133 g.<sup>5</sup> During the digestion trial, they consumed 11.8 g protein per 100 g live weight. The next 14 coefficients were obtained with seven animals in two trials. In one trial (*e*, Table 3) their weight ranged from 90 to 119 g (average 101 g) and they consumed 12.8 g protein per 100 g live weight. In the second trial (*f*, Table 3) their weight ranged from 106 to 132 g (average 120 g) and the consumption of protein was 11.8 g per 100 g live weight.

All the other coefficients shown in Table 3 (except the first three for Holstein milk) were obtained with stock animals which weighed, as a rule, from 175 to 300 g. The last nine coefficients under Brown Swiss (*h*) were obtained with stock animals weighing 177 to 321 g (average 235 g.). They consumed 9.6 g protein per 100 g live weight.

In the light of these data, it is reasonable to assume either that the young growing animal, on account of its greater requirement of protein for growth, absorbs the protein of the food more completely than the mature animal, or that, because of its larger food intake in proportion to body weight, it suffers a loss of endogenous nitrogen in the feces which is slightly less in proportion to body weight than is the

<sup>5</sup> g is the abbreviation for gram or grams.

case with the large, mature animal. It is also possible that both factors may be operative, although the first is probably the more important.

Were the 18 coefficients shown under Brown Swiss (Table 3) to be omitted, the mean values for the breeds would agree more closely.

The coefficients shown in Table 3 under (a), (b), and (c) for both the Ayrshire milk and the Holstein milk are those obtained in three successive trials in which the experimental procedure was modified as already explained. In trial (a), the feces which showed evidences of urine contamination were washed, but in trials (b) and (c) no washing was done. Previous to trial (c) the animals were clipped. This comparison, although limited in extent, tends to substantiate the soundness of the procedure used in all of the earlier trials.

The apparent digestibility of the total solids of the milk was found to be slightly higher than that of the protein. (Table 4.) As in the case of fat and total protein, the differences between breeds in apparent digestibility of the total solids are so small that it is very probable that no true differences exist. In round numbers, the means of the coefficients for each of three breeds are 92, and for the other two breeds, 93. The other statistical constants also agree closely.

TABLE 4.—Comparative apparent digestibility of the total solids of the milk of dairy cattle \*

	Summary of coefficients of apparent digestibility of total solids				
	Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
	90.6	93.8	90.6	91.7	92.3
	92.0	94.7	92.4	91.0	91.8
	92.5	95.0	92.8	91.2	94.1
	93.7	95.2	92.1	91.8	91.8
	95.0	93.9	94.4	91.8	91.8
	91.9	91.3	91.5	90.9	89.9
	91.5	95.4	90.7	93.1	92.7
	92.6	94.5	92.1	90.9	92.0
	92.0	94.1	92.3	92.0	91.0
	95.8	94.4	90.8	90.6	91.1
		95.2		91.9	
		95.6		93.1	
		93.9		92.5	
		95.4		91.7	
		92.1		93.7	
		92.2		90.2	
		93.7		92.4	
		92.5		90.5	
		92.7		91.2	
		92.7		93.3	
		92.4			
		92.7			
		92.7			
		91.4			
		92.1			
		92.1			
		91.5			
		93.0			
		92.6			
		91.9			
		92.8			
		92.0			
		92.3			
		90.8			
Mean.....	92.8±0.33	93.3±0.15	92.0±0.23	91.8±0.15	91.9±0.22
Standard deviation.....	1.5±.23	1.3±.11	1.1±.17	1.0±.10	1.1±.16
Coefficient of variability.....	1.6±.25	1.4±.12	1.2±.18	1.1±.11	1.1±.17

\* Number of determinations: Ayrshire, 10; Brown Swiss, 34; Guernsey, 10; Holstein-Friesian, 20; Jersey, 10.

The feces of 14 rats fed Brown Swiss milk and of 10 rats fed Holstein milk were tested carefully for sugar, both Fehling's and Benedict's methods being used. All these tests were negative. Similar results had been secured in previous trials with two brands of evaporated milk and two brands of dried milk (19, 20). It is believed, therefore, that the sugar of fresh whole milk is completely digestible.

The fat, protein, and total solids of the milk fed during the digestion trials are shown in Table 5. Each value given represents the average of two or more determinations made upon a composite sample.

TABLE 5.—*Composition of milk fed during digestion trials*

Breed	Fat	Protein	Total solids	Breed	Fat	Protein	Total solids
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Ayrshire.....	3.04	3.35	12.15	Guernsey.....	4.35	3.70	13.79
	3.35	3.35	12.15		2.95	2.97	14.02
	3.53	3.26	12.21		2.95	2.97	10.60
	3.26	3.90	12.64		3.26	3.26	10.34
	2.90	3.86	12.17		2.93	2.93	11.75
	2.35	3.79	12.47	Holstein.....	2.77	2.99	11.65
	3.65	3.86	11.80		2.77	2.99	11.28
	3.86	3.79	13.02		2.93	2.93	11.15
	3.79	3.89	13.72		2.76	2.76	11.04
	3.91	4.14	13.84		3.64	3.64	11.02
Brown Swiss.....	4.02	4.15	13.89	Jersey.....	5.08	5.08	13.74
	4.33	4.15	14.12		5.08	5.08	14.96
	2.83	3.88	14.12		5.08	5.08	14.96
	2.83	3.44	12.34		5.08	5.08	14.96
	2.69	3.50	11.66		5.08	5.08	14.96
	4.14	4.15	13.60		5.08	5.08	14.96
	3.29	4.15	13.05		5.08	5.08	14.96
	3.08	4.15	13.22		5.08	5.08	14.96
			11.74				

## SUMMARY

The apparent digestibility of the fresh whole milk of five breeds of dairy cattle was studied by means of 10-day digestion trials with albino rats. Milk only was fed.

The fat of fresh whole milk was found to be very highly digestible, the coefficients obtained indicating an apparent digestibility of about 99 per cent. There were no significant differences in digestibility of the milk fat of the breeds studied.

The protein of fresh whole milk was found to be about 91 per cent digestible. The data show slight differences between the milks of the breeds, but on account of some variations in the methods employed at different times in the course of the investigation, these differences between breeds are believed to have no significance.

The total solids of fresh whole milk were slightly higher in digestibility than the protein, as would be expected, but no true differences between breeds were found.

The sugar of fresh whole milk was found to be completely digestible.

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# THE CARBON METABOLISM OF *FUSARIUM OXYSPORUM* ON GLUCOSE<sup>1</sup>

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## INTRODUCTION

A very important phase of research in plant pathology is that which deals with the physiological relationships existing between the causative organism and the host. Any information on the biochemistry of the causative organism should prove of value in studying the physiology of a plant disease. It is very probable that some metabolic product of the organism is the important factor producing the symptoms of the disease in the plant.

## REVIEW OF LITERATURE

In the past considerable work has been done on the biochemistry of *Fusarium lini* Bolley, the organism causing flax wilt. In 1920 Tochinali (9, 10, 11)<sup>2</sup> reported a rather complete study of the food requirements of this organism. In 1924 Anderson (1) repeated much of the work of Tochinali and also studied the products of metabolism of this organism on glucose and xylose media. He found that the organism produced a rather typical alcoholic fermentation on glucose. About 90 per cent of the original carbon could be accounted for in the products of metabolism studied. In the case of xylose about 80 per cent of the original carbon could be accounted for in the ethyl alcohol, carbon dioxide, and mycelium produced. He suggested the possibility that the alcohol caused the wilt.

In 1926 Letcher and Willaman (6) attempted to correlate the pathogenicity of various strains of *Fusarium lini* with their ability to produce ethyl alcohol. They examined nine strains of the fungus and found that the two which were the least virulent on flax produced the least alcohol. It should be noted, however, that the strain which produced the most alcohol was not the most virulent.

In 1928 White and Willaman (14, 15) studied the fermentation of pentoses by *Fusarium lini* and accounted for nearly 100 per cent of the original carbon in the form of carbon dioxide, ethyl alcohol, and mycelium. They also reported the growth of the organism on the postulated intermediate products formed by yeast in the pyruvic acid theory of fermentation. They believed that *F. lini* ferments glucose by the same mechanism as that of yeast.

Reynolds (8) in 1926 reported on the utilization of various carbohydrates and nitrogen compounds by *Fusarium lini*.

In 1931 Birkinshaw, Charles, Raistrick, and Stoye (2) reported on the carbon metabolism of various species of *Fusarium*, including *F. oxysporum*, grown on a glucose medium. They found some variation among the various species but all of them produce ethyl alcohol.

<sup>1</sup> Received for publication July 22, 1932; issued April, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 481.

## PURPOSE OF THE INVESTIGATION

The purpose of the present work has been to open the way for an investigation of another wilt-producing fungus, *Fusarium oxysporum* Schlecht., using the same type of medium and employing essentially the same methods of determining the products of metabolism as were employed by Anderson (1) on *F. lini*. This has made it possible to compare the metabolism of these two organisms under practically the same conditions. The principal part of the study has been to determine the quantitative distribution of the chief products of metabolism (ethyl alcohol, carbon dioxide, and mycelium) of *F. oxysporum* on glucose. A further study has been made to determine whether or not ethyl alcohol, in concentrations equal to that produced by *F. oxysporum* in artificial cultures, is toxic to potato cuttings.

## METHODS

## SOURCE OF FUSARIUM OXYSPORUM CULTURE

The culture of the organism used in this work was obtained from the mycological herbarium of the University of Minnesota through the courtesy of Dr. Louise Dosdall. This was isolated in 1919 by Dr. G. R. Bisby (3) in connection with his studies on the *Fusarium* diseases of potatoes and truck crops.

## CULTURE MEDIA

The mineral medium used in this work was the same as that used by Tochinal (9) and Anderson (1) in their work on *Fusarium lini*. It has the following composition:

Ammonium nitrate.....	1.00 g <sup>3</sup>
Magnesium sulphate.....	.25 g
Monopotassium phosphate.....	.50 g
Water to make.....	1,000 c c

To this stock solution sufficient glucose was added to make an approximately 2 per cent solution of glucose. The exact quantity of glucose was determined after sterilization by the Folin-Wu (5) method.

## HYDROGEN-ION CONCENTRATION

Hydrogen-ion concentration determinations were made by the electrometric method using the quinhydrone electrode.

## CULTURE FLASKS AND TEMPERATURE OF INCUBATION

The culture flasks used were 500 c c Erlenmeyer flasks fitted with 2-hole rubber stoppers. Glass tubes were placed through the holes of the stoppers and bent at right angles just above them. One of the tubes extended just below the stopper and the other reached to the bottom of the flask. The outer end of each tube was plugged with cotton and fitted with a rubber tube that could be closed by a pinch-cock. Into each flask 300 c c of the medium were placed and the long tube was drawn up so that the lower end was about an inch above the surface of the liquid. The flasks were then sterilized at 15 pounds pressure for 20 minutes. When cooled each flask was inoculated by the addition of 5 c c of a spore and mycelium suspension of the

<sup>3</sup> g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

organism in water. The stoppers were then adjusted and sealed with paraffin and the rubber connections closed with pinchcocks.

The flasks were kept at room temperature in a laboratory where the temperature varied only slightly from 25° C.

#### DETERMINATION OF CARBON DIOXIDE

Carbon dioxide was determined at frequent intervals throughout the experiment in order to prevent loss of this product of metabolism. The method used was to aerate each culture flask into barium hydroxide solution contained in a Truog (12) tower fitted into a 500 c c suction flask through a rubber stopper. The air used for aeration was freed from carbon dioxide by passing it through soda lime. In order to prevent loss of alcohol, a Truog tower containing concentrated sulphuric acid was placed ahead of the barium hydroxide tower. A separate sulphuric acid tower was prepared for each culture flask. These were used every time a culture flask was aerated for carbon dioxide and finally the alcohol was determined, as will be described later. After aeration the excess of barium hydroxide was titrated with standard hydrochloric acid solution using phenolphthalein as an indicator. Before flasks were removed for final analysis the long tube was pushed below the surface of the liquid and aerated for 30 minutes in order to remove the last traces of carbon dioxide. Each time the flasks were aerated for carbon dioxide a blank determination was made. The titer of the carbon dioxide determinations was subtracted from this blank and the difference used in calculating the carbon dioxide in the sample.

#### DETERMINATION OF DRY MATTER AND CARBON IN THE MYCELIUM

When the experiment was started a sufficient number of culture flasks were prepared so that duplicate samples could be analyzed at intervals of a week or 10 days over a period of about two months. The weight of mycelium was determined by filtering through a weighed Gooch crucible and washing with water. The pad of mycelium was then dried to constant weight at 100° C. The filtrate was made up to a volume of 500 c c and aliquot portions were used for subsequent determinations.

The carbon in the mycelium was determined by the wet combustion method using the Knorr apparatus and the details of procedure as outlined by White and Holben (13). The entire mycelium mat, together with the asbestos, was introduced into the digestion flask.

#### DETERMINATION OF ALCOHOL

For the determination of ethyl alcohol the method of Dox and Lamb (4) was used with modifications. In this method the alcohol is oxidized to acetic acid which is distilled and titrated with standard alkali. After filtering and washing the mycelium and making up the filtrate to a volume of 500 c c, a 300 c c aliquot of the filtrate was saturated with solid ammonium sulphate and the mixture aerated for 48 hours into a Truog tower containing concentrated sulphuric acid. The acid mixture, together with the glass beads, was then transferred to a 2-liter Claisen flask containing 22 g of potassium dichromate. After standing for 20 minutes the resulting acetic acid was distilled and titrated with standard alkali. In this distillation the flask was

heated until foaming occurred. Next 100 c c of carbon-dioxide-free water were added to the distilling flask, the distillation was repeated, and the distillate titrated. This last procedure was repeated until a constant titer was obtained. This represents the amount of sulphuric acid unavoidably distilled at each distillation, and the constant titer times the number of distillations represents a blank which must be subtracted from the sum of all the titrations.

Since in the determination of carbon dioxide a sulphuric acid tower was always placed ahead of the barium hydroxide tower to collect any alcohol given off, another alcohol determination was made on the contents of this tower. The quantity of alcohol found was added to that found in the filtrate to give the total alcohol produced in a given flask.

#### DETERMINATION OF GLUCOSE

Glucose was determined by the method of Folin and Wu (5). An aliquot of the filtrate from the mycelium determination was diluted so that 1 c c contained about 0.1 mg of glucose. Under these conditions the color of the unknown matches closely that of the Folin and Wu dilute glucose standard.

#### PRESENTATION OF DATA

Sixteen flasks with 300 c c of medium containing a known quantity of glucose as the only source of carbon were inoculated with a spore and mycelium suspension of *Fusarium oxysporum*. These were stoppered and the aeration tubes were closed by means of rubber tubes and pinchcocks in order to prevent loss of carbon dioxide. Carbon dioxide was determined at frequent intervals. During the active growth of the organism it was determined daily. At intervals of a week or 10 days two flasks were removed and analyzed for residual carbon dioxide, carbon in the mycelium, glucose, and ethyl alcohol. The original culture medium was analyzed for glucose and the pH determined. The last two flasks were used for pH determinations.

TABLE 1.—Distribution of metabolic products of *Fusarium oxysporum* on a glucose medium at various stages of growth

Age of culture (days)	Mycelium				Carbon dioxide		Ethyl alcohol		Glucose		Total recovery of carbon as related to initial carbon <sup>a</sup>	pH
	Weight	Weight of carbon	Carbon	Carbon as related to initial carbon <sup>a</sup>	Weight of carbon	Carbon as related to initial carbon <sup>a</sup>	Carbon in 300 c c	Carbon as related to initial carbon <sup>a</sup>	Carbon in 300 c c	Carbon as related to initial carbon <sup>a</sup>		
	Gram	Gram	Per cent	Per cent	Gram	Per cent	Gram	Per cent	Gram	Per cent	Per cent	
0.....									2.0633	100	100	
12.....	0.1399	0.0651	46.53	3.15	0.1998	9.68	0.3501	16.97	1.6170	78.37	108.17	4.15
20.....	.2466	.1129	45.78	5.47	.5150	24.96	1.0153	49.21	.0141	.68	80.33	
27.....	.2432	.1095	45.02	5.31	.5389	26.12	1.0172	49.30			80.72	
36.....	.2484	.1140	45.89	5.52	.5457	26.45	<sup>b</sup> 1.0379	50.30			82.23	
46.....	.2440	.1118	45.82	5.42	.5779	28.01	<sup>b</sup> 1.0707	51.89			85.32	
59.....	.2399	.1204	50.18	5.83	.6376	30.90	1.0030	48.61			85.34	
68.....	.1929	.1027	53.24	4.98	.7051	34.17	.9878	47.88			87.03	6.55

<sup>a</sup> Expressed as percentage of the carbon in the glucose originally present.

<sup>b</sup> I determination.

In all, four experiments were run. In Table 1 and Figure 1 the data are given for one representative experiment. It should be noted that results are expressed in terms of carbon and also in terms of percentage of the carbon of the glucose originally present in each flask.

Referring to Table 1 and Figure 1, the following facts are brought out concerning the progressive changes in the products of metabolism.

#### THE MYCELIUM AND ITS CARBON CONTENT

The weight of mycelium increases rapidly, reaches a maximum at 36 days, and finally decreases. The maximum is reached at the time that all the glucose has been consumed. Apparently stored material

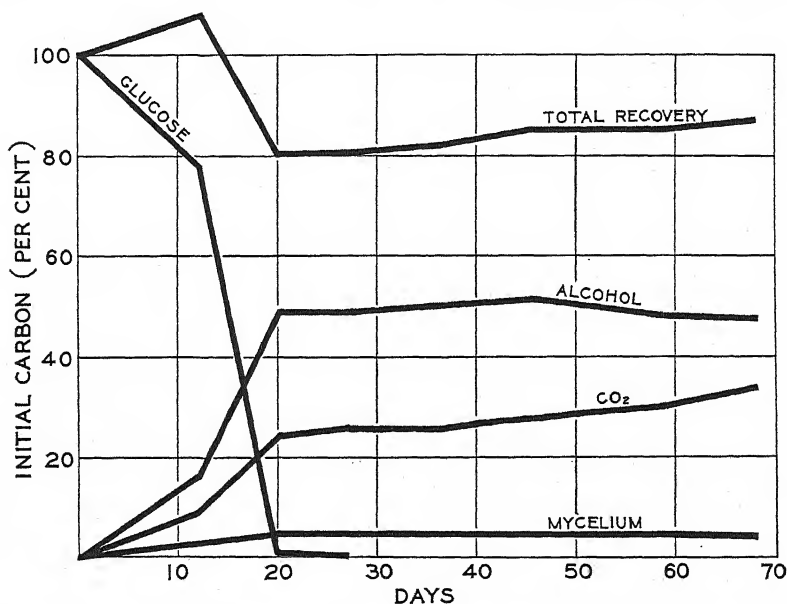


FIGURE 1.—Distribution of metabolic products of *Fusarium oxysporum* when grown on glucose

in the mycelium is consumed after other sources of food have been utilized.

The weight of dry mycelium produced approximates that found by Anderson (1) for *Fusarium lini*, although he used only one-third as much medium. He found as high as 16.75 per cent of the original carbon in the mycelium, while with *F. oxysporum* 5.83 per cent is the maximum. *F. lini* shows a very definite utilization of ethyl alcohol for mycelium production. *F. oxysporum* is apparently unable to utilize ethyl alcohol for mycelium building purposes.

The percentage of carbon in the mycelium remains very constant at about 46 per cent for 46 days when it increases to a final value of over 53 per cent. This increase in carbon content of mycelium with age was also noted by Anderson (1) in his work on *Fusarium lini*. Since carbohydrates contain about 40 per cent of carbon, proteins from 51 to 55 per cent, and a typical fat such as tristearin 76.87 per cent, Anderson believes that the increase of carbon in the mycelium indicates

a change of the carbohydrate in young mycelium into fat as the mycelium ages. Since the percentage of nitrogen in the mycelium decreased with age, he felt there was not a change of carbohydrate to protein. Although nitrogen was not determined on the mycelium in this work on *F. oxysporum*, it is a reasonable assumption that the increase in carbon content is due to a conversion of carbohydrate into fat.

The percentage of original carbon found in the mycelium is well over 5 during active growth. In the last analysis this drops to 4.98 per cent, which indicates a utilization of carbon in the mycelium for food purposes as other sources of food are consumed.

#### CARBON DIOXIDE

Carbon dioxide is produced throughout the experiment. During the early part of the experiment carbon dioxide was produced very rapidly, necessitating its determination daily. When the glucose is all consumed, the rate of carbon dioxide production decreases so that it is necessary to determine it only at infrequent intervals. The percentage of original carbon in the form of carbon dioxide increases throughout the experiment, reaching a maximum of 34.17 per cent in the final analysis.

#### ETHYL ALCOHOL

Ethyl alcohol is produced rapidly by *Fusarium oxysporum*. In 20 days the percentage of original carbon in the form of ethyl alcohol is 49.21. This increases gradually to 51.89 per cent at the end of 46 days. From then on the quantity of alcohol decreases, falling to 47.88 per cent of the original carbon at the end of the experiment. Anderson (1) in his work on *F. lini* found that this organism converted 52.75 per cent of the original carbon into alcohol when the amount of alcohol was at a maximum. At the end of the experiment the original carbon in the form of ethyl alcohol was only 29.51 per cent. *F. lini* very definitely utilizes ethyl alcohol as a food material. There is only slight utilization of ethyl alcohol by *F. oxysporum*.

#### GLUCOSE

The glucose of the medium is consumed rapidly. In 20 days there is only a trace left. Since the total recovery of carbon at the end of the 12-day period is 108.17 per cent, it is felt that the error is in the determination of glucose. It is very possible that in the early stages of growth the organism converts glucose into intermediate products which have the power of reducing copper solutions. If this is true, the value for glucose at the 12-day period is high.

#### TOTAL RECOVERY OF CARBON

In the column headed "total recovery" in Table 1 are recorded the sum of the values obtained for the various products of metabolism. It will be noted that at the end of the 12-day period the recovery is 108.17 per cent. As pointed out above this is likely due to the production of intermediate products which reduce copper solutions, rendering the glucose determination high. At the end of the 20-day period the total recovery has fallen to 80.33 per cent. From then on the total recovery gradually increases until at the end of the experiment it is 87.03 per cent. The fact that all of the carbon is not

accounted for indicates that there are other products of metabolism than those studied, although some of the loss may be due to incomplete recovery of some of the products studied.

#### HYDROGEN-ION CONCENTRATION

Determinations of pH were made only at the beginning and at the end of the experiment. The initial pH was 4.15 and the final pH was 6.55. Anderson (1) in his work on *Fusarium lini* found that this organism changed the pH of the medium toward the optimum for growth. It appears that the same occurs with *F. oxysporum*.

#### RATIO OF PRODUCTS OF METABOLISM TO ONE ANOTHER AND TO GLUCOSE CONSUMED

The relationships existing between the products of metabolism and the compounds consumed by a fungus have been expressed by means of various ratios and percentages. Such terms as respiration coefficient, economic coefficient, respiration equivalent, plastic equivalent, etc., have been introduced into the literature. There has been such a lack of uniformity with regard to the usage of these terms that in Table 2 a formula is placed at the head of each column to make perfectly clear the sense in which these terms are used in this discussion. They are used in the same sense as used by Peterson, Fred, and Schmidt (?).

The respiration coefficient represents the grams of carbon dioxide produced per gram of dry mycelium. It will be noted from Table 2 that this coefficient gradually increases with the age of the culture. Finally there is a rapid increase due to the consumption of the mycelium itself as a food material. The values are very much higher than those found by Anderson (1) for *Fusarium lini*.

TABLE 2.—Quantitative relationships existing between the various metabolic products of *Fusarium oxysporum* when grown on a glucose medium

Age of culture (days)	Respiration coefficient, weight of CO <sub>2</sub> weight of mycelium	Economic coefficient, weight of sugar consumed weight of mycelium	Respiration equivalent, of CO <sub>2</sub> ×100 carbon consumed	Plastic equivalent, carbon of mycelium×100 carbon of glucose consumed	Alcohol equivalent, carbon of alcohol ×100 carbon of glucose consumed	Carbon of alcohol carbon of CO <sub>2</sub>
12.....	5.24	7.98	44.76	14.60	78.45	1.75
20.....	7.66	20.78	25.03	5.51	52.09	1.97
27.....	8.13	25.32	26.12	5.31	49.30	1.88
36.....	8.06	24.79	23.45	5.52	50.30	1.90
46.....	8.68	25.24	23.01	5.41	51.90	1.85
59.....	9.75	25.66	30.90	5.83	48.61	1.57
68.....	13.40	31.92	34.17	4.98	47.90	1.40

The economic coefficient represents the grams of glucose necessary to produce 1 g of dry mycelium. This coefficient increases with the age of the culture, reaching a maximum of 31.92 in 68 days. Anderson (1) found a maximum value of 13.1 with *Fusarium lini*.

The respiration equivalent is the percentage of carbon consumed that is transformed into carbon dioxide. With the exception of the 12-day period, where the value is high due to a false sugar value, this

equivalent increases with age. Anderson (1) found slightly higher values for *Fusarium lini*.

The plastic equivalent represents the percentage of carbon source used in mycelium synthesis. Here again is found a high value at the 12-day period due to a false sugar value. The other values are approximately constant but lower than those found by Anderson (1) for *Fusarium lini*.

The alcohol equivalent is a new term that the authors have introduced which expresses the percentage of carbon of the glucose consumed that is converted into alcohol. At the end of the 12-day period the value is 78.45 which is undoubtedly too high due to a false glucose value as mentioned above. For the remaining periods the value drops to around 50. In the last two periods the value drops due to consumption of the alcohol by the fungus.

In the last column the ratio of carbon in alcohol to carbon in carbon dioxide is given. In a typical alcoholic fermentation glucose is decomposed according to the following equation:



In this equation the ratio of carbon in alcohol to carbon in  $\text{CO}_2$  is 2:1. It will be seen from Table 2 that at the end of the 20-day period the value is 1.97:1, which is very close to the theoretical for a typical alcoholic fermentation. From this point on the ratio decreases until at the end of the experiment it is 1.40:1. Here again utilization of the alcohol by the fungus is indicated.

In the early part of this study an error was made in making up the nutrient mineral solution. In place of 1 g of  $\text{NH}_4\text{NO}_3$  per liter, 100 g per liter were used. It is felt worthy of mention that *Fusarium oxysporum* grew on this medium and produced a typical alcoholic fermentation. When the distribution of the products of metabolism were charted as in Figure 1, in general, the curves were the same. The main difference between the two figures was that the rate of production of the various products of metabolism on the strong mineral solution was about one-third as fast as on the weak mineral solution.

#### THE EFFECT OF ALCOHOL ON POTATO CUTTINGS

Anderson (1) has suggested that the toxicity of *Fusarium lini* to flax may be due to the production of ethyl alcohol by the organism in the plant tissue. When it was ascertained that *F. oxysporum* produces ethyl alcohol on glucose it became of interest to study the effect of solutions of ethyl alcohol at various concentrations on potato cuttings.

Solutions of alcohol were made, varying in concentration from 0.5 to 5 per cent in increments of 0.5 per cent. Potato cuttings were placed in these solutions in beakers. Several cuttings were placed in tap water as a control. In no case was there any appreciable change within 24 hours, other than a slight curling of the leaves. After 48 hours a mottled yellow began to appear on the leaves of the cuttings in alcohol. This did not occur in the controls. The stems remained firm. When the cuttings in alcohol were shown to a pathologist familiar with potato diseases, he believed they were affected with an extreme case of leaf mosaic. There was no wilting of the stems even



at the end of five days. A very striking odor resembling that of ripe cantaloupes was given off by the cuttings in alcohol.

Since in all of the above work the stems remained firm, it was decided to determine the concentration of alcohol necessary to produce definite and rapid wilting. Cuttings were placed in solutions containing from 10 to 55 per cent of alcohol in increments of 5 per cent. Within two hours there was a general wilting in all cases, the wilting increasing in severity with the higher concentrations. There was no appreciable change in color of the leaves as was noted with the more dilute solutions of alcohol. The cantaloupe odor previously noted was very strong in all cases. Judging from the esterlike odor, one is led to the hypothesis that the potato plant is able partly to protect itself against ethyl alcohol by converting it to an ester.

The findings in solutions of alcohol up to 5 per cent are in agreement with the work of White (16) who observed no ill effects on tomato cuttings in solutions of ethyl alcohol up to 4 per cent.

### SUMMARY AND CONCLUSIONS

It is evident from the data presented that the main products of metabolism of *Fusarium oxysporum* on glucose are carbon dioxide and ethyl alcohol. The proportion of these two compounds formed indicates that the organism causes a rather typical alcoholic fermentation. In this respect *F. oxysporum* is similar to *F. lini*.

*Fusarium lini* very definitely uses ethyl alcohol as a source of food supply while *F. oxysporum* makes only slight use of this product.

Work with potato cuttings in ethyl alcohol solutions would indicate that this product is not responsible for the wilting of potato plants. It is likely that small quantities of alcohol are rendered nontoxic by conversion into an ester by the potato plant. Potato cuttings in a 10 per cent alcohol solution showed very definite symptoms of wilting. It is quite possible that in this case the concentration of alcohol in the tissue was much less than 10 per cent. Although there is no experimental evidence to support the theory that alcohol is responsible for the wilting, it is reasonable to suppose that alcohol may at least be a factor in the production of the symptoms of wilt in potato plants infected with *Fusarium oxysporum*.

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## INFLUENCE OF LIGHT, TEMPERATURE, AND SOIL MOISTURE ON THE HARDENING PROCESS IN ALFALFA<sup>1</sup>

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### INTRODUCTION

Although much progress has been made in increasing the ability of plants to withstand cold by selection and breeding, little is known of the fundamental physiological phenomena which serve to make a plant "winter hardy." In fact, these improvements have been made by plant breeders under the handicap of having to wait for "test" winters to eliminate the unhardy selections. In any improvement program involving winter hardiness a simple method of determining cold resistance would be of great value, for with such a method newly introduced varieties could be tested without delay.

Studies of cold resistance with the aid of controlled freezing methods have gone forward in various laboratories with promising results. Some workers in this field, notably Hill and Salmon (7),<sup>3</sup> Akerman (1), and Martin (11), have suggested that it is essential for plants to go through the hardening process before correct varietal differentiation in cold resistance can be obtained. The influence of such factors as light, moisture, and even temperature on the ability of plants to resist cold is, however, still largely unknown. Very little is known regarding the time necessary for optimum hardening under uniform conditions. A better understanding of these and other factors would lead to greater refinements in the procedure of determining hardiness and might well be made one of the first steps in a study of the whole problem of winter hardiness.

The present paper deals with the influence of light, temperature, and soil moisture on the ability of alfalfas to survive cold. In practically all the tests several varieties<sup>4</sup> having a wide range in winter

<sup>1</sup> Received for publication Mar. 30, 1932; issued April, 1933. Presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1931. This paper, the third of a series on hardiness in alfalfa, is based on cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology, Nebraska Agricultural Experiment Station.

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<sup>3</sup> Reference is made by number (italics) to Literature Cited, p. 514.

<sup>4</sup> It is recognized that the term "variety" is not used in the correct botanical sense. However, since the alfalfas employed in this study have distinct physiological differences and since the term is convenient, they will be termed "varieties" throughout this paper.

hardiness were used with the object of determining what particular set of environmental conditions would bring out the greatest varietal differentiation.

The effect of low temperature on plants has been studied for at least 200 years, and voluminous literature on the subject has accumulated. The work of previous investigators has been reviewed by Blackman (2), Chandler (3), Harvey (5), Rosa (22), Akerman (1), Maximov (12), Steinmetz (24), and others, and therefore only the more pertinent papers will be mentioned in connection with the discussion of each environmental factor.

## MATERIALS AND METHODS

### EQUIPMENT

All results herein reported were obtained from seeds sown and from plants grown, hardened, frozen, and thawed in the greenhouse. The equipment used in the plant pathology greenhouses of the Nebraska Agricultural Experiment Station has been described in detail by Peltier (17). Briefly, the greenhouse, besides having the ordinary warm space maintained at a temperature of approximately 20° C., is equipped with a hardening room, which is cooled by air drawn over artificially refrigerated coils. The hardening room is similar to the warm greenhouse except that it has double-glass top and sides, with a 2-inch air space between the glasses. It can be maintained at a temperature varying between 0° and 5°.

Six temperature cases described by Peltier and Goss (18) were placed at one side of the hardening room. These were electrically heated and could be maintained fairly constant at any desired temperature above the room temperature. The variation in temperature in these cases was less than  $\pm 1^\circ$  C. A current of air was forced through the cases constantly by means of an electric blower connected through a common conductor to each case. This blower had a capacity of 225 cubic feet per minute, and since the volume of all the cases was only 252 cubic feet (42 cubic feet each) there was a change of air approximately every  $1\frac{1}{8}$  minutes. Actually the change may not have been so rapid, since there was not a free movement of air through the cases. However, sufficient air passed through to keep the temperature constant. Two and one-half feet above one case was hung a 1,000-watt electric-light bulb, used for the long-day experiments. For the 17-hour day, the light was put on at 4 p. m. and was automatically switched off at the desired time by means of an alarm clock, the alarm winder of which was tied to a switch. No artificial light was used in the morning, so the light went off regularly at 10 to 12 o'clock midnight, as desired. In addition to the six cases mentioned, three cases at approximately 20° C. were placed in an adjoining warm room. These cases were used in preference to the ordinary greenhouse, as conditions in them were more nearly comparable to conditions in the cases in the hardening room.

The head house was equipped with a freezing room 8 by 7 by 8 feet, which could be maintained by artificial cooling at a temperature as low as  $-35^\circ$  C. with a variation of  $\pm 2^\circ$ .

The containers in which the plants were grown were of three types: Wooden flats, 15 by 18 by 7 inches; metal water-tight containers, 4 by 4 inches by 6 inches deep; and ordinary porous clay pots, 4 inches

in diameter. The seed was sown in rows in the flats, each flat containing 5, 6, or 7 rows with approximately 30 plants per row. The seed was sown broadcast in the pots and tin containers, each containing from 10 to 20 plants. No effort was made to have the number constant, as preliminary experiments indicated that a slight difference in the number did not introduce an appreciable error. Difficulties due to damping off of young plants during the short days in winter were largely overcome by wetting the soil in the pots thoroughly before planting, covering the seed with a shallow layer of sand, and supplementing daylight with powerful electric lamps for a portion of the night.

The soil used throughout the experiments was a rich loam mixed with sand in the proportion 4:1.

#### VARIETIES USED

In practically all the work three to six varieties of alfalfa were used, including the following: Turkestan, F. C. I.<sup>5</sup> No. 15754; Grimm, F. C. I. No. 15713; Nebraska common; Utah common, F. C. I. No. 15815; Arizona common, F. C. I. No. 15837; and Ladak, F. C. I. No. 14135. If only three were included, they were usually Turkestan, Grimm, and Arizona common. In a few of the later experiments Grimm alone was used. The first year the seed was treated with concentrated sulphuric acid for 10 minutes before it was sown. This was to insure against "hard" seeds coming up after freezing. With more experience it was found that new seedlings could readily be distinguished, and, except in a few instances, treating was discontinued the second year.

#### MEASUREMENT OF HARDENING

The response of a plant to the hardening process was measured by its ability to survive cold in the artificial freezing chamber. Except where specially noted, the technic followed in all the experiments herein reported, which in all instances was based on previous experiments, has been described by Peltier and Tysdal (20). The main features common to all tests were as follows: 12 to 16 hours before exposure in the freezing room the porous pots or flats were saturated with water. Natural drainage left the soil in different pots or flats at approximately the same moisture content. During freezing the temperature in the freezing room was maintained as nearly uniform as possible. Relatively short exposures at rather low temperatures were employed. Immediately after freezing, the plants were transferred to a warm room, where they were allowed to recover.

In all experiments the measure of survival of the plants was based on actual counts. The plants were counted before they were put into the hardening room and again two weeks after they were frozen. From these two numbers the percentage survival was calculated. In practically all the experiments the freezing was so severe that the tops of the plants were completely killed, and the surviving plants had, therefore, to start new growth from the crown. These plants showed a great difference in the rate of recovery, depending on the degree of injury. An estimate of the degree of injury might therefore

<sup>5</sup> The letters F. C. I. indicate accession numbers of the Division of Forage Crops and Diseases.

have been used as a basis for tabulating differences. This method has been used by Hill and Salmon (7), Quisenberry (21), and others, but since it is subject to errors of judgment, it was considered more accurate, in the case of alfalfa at least, to make an actual count of the plants surviving.

#### AGE OF PLANTS AT FREEZING

Steinbauer (23) found red-clover plants to be least resistant to cold at 21 days of age, when they were forming the first pair of permanent leaves. In alfalfa, however, Peltier and Tysdal (20) found that resistance began earlier and increased with age up to 60 days, except that seedlings 5 days old, just emerging from the soil, were more resistant than those 10 days old with two cotyledons and the third leaf showing. The relative varietal response at 25 to 30 days was the same as at 53 or 60 days. As a result of these findings, the plants used in the following studies were allowed to grow in the warm greenhouse approximately 30 days, and unless otherwise stated this procedure was followed throughout.

#### LENGTH OF TIME IN HARDENING ROOM

Peltier and Tysdal (20), in studying the influence of length of the hardening period on resistance to cold, kept alfalfa plants in the hardening room at temperatures of 2° to 4° C. from 1 to 35 days. They found that resistance increased with increasing time in the hardening room up to 14 to 15 days. Thereafter it remained much the same up to 23 days; at 35 days it was again low, probably due to exhaustion of the plants. This decrease in resistance corresponds with observations by Molisch (13), who found that plants continuously exposed to a temperature too low for normal metabolism, but above the freezing point, die. From the results of these experiments it was concluded that a 14-day period would be optimum for hardening under constant conditions. It is realized that this period may not be best under all conditions, but it affords a basis for standardizing the experiments in this respect, without which it would be impossible to deal satisfactorily with the large number of variables involved.

#### INFLUENCE OF LIGHT ON THE HARDENING PROCESS

To determine the effect of light on the hardening process in the different alfalfas, three groups of experiments were made, namely, those primarily concerned with (1) the influence of length of day, (2) the influence of light intensity, and (3) the influence of wave length of light.

##### INFLUENCE OF LENGTH OF DAY

In general, the results obtained by investigators of the relation of light to plant growth point to decreased growth and increased chlorophyll concentration with decreased light intensity. Low light intensities favor top growth at the expense of root growth, although on this point there is some difference of opinion. Greater storage, or at least greater manufacture, of carbohydrates takes place in the long-day plants. Small variations in the quality of light do not markedly affect plant growth, but optimum growth occurs under a complete spectrum rather than under a portion of it. Length of day exhibits a more striking influence on development of plants than does intensity of light.

Oakley and Westover (16) clearly demonstrated that the prostrate habit exhibited by some species and varieties of alfalfa under shortened day length in the fall might be traced to their response to light. However, they did not show whether this response to light actually served to harden the plant.

The present study was undertaken to determine the part played by length of day in the hardening process in alfalfa varieties. In fact, this work was started with the assumption that a long day would build up carbohydrate reserves in the plant, and results reported by Graber et al. (4), Willard (25), and others indicated that large carbohydrate reserves better enable plants to overwinter successfully. From this it appeared that the longer the day, within certain limits, the better chance the plant would have of surviving low temperatures. As will be seen, this hypothesis proved to be incorrect.

The experiments to determine the influence of light on the hardening process were made in the cases in the hardening room and adjoining warm room. These cases were large enough to contain thirty-two 4-inch clay pots. Rubberized light-proof hoods were made to fit over the individual cases and were used to exclude light from those in which the short-day plants were placed, while artificial light from a 1,000-watt Mazda C lamp placed 2 feet above the case was used to supplement ordinary daylight for the long-day plants. The cases could be maintained at any temperature above the hardening-room temperature of about 5° C. They were aerated by a continuous stream of air from a blower connected through a common duct to all cases.

The experiments generally were planned so that three cases were regulated to as nearly the same temperature as possible, and the length of day varied between these three cases. In only two experiments did the average temperature vary more than 0.5° C. between different cases, and in these two experiments only slightly more than 0.5°. The 17-hour day was made up of the normal light plus sufficient artificial light to make up 17 hours of continuous light. In practically all the experiments both a day of normal length for the particular period of the year and a 17-hour day were used for comparison with a 7-hour day. The 7-hour day, unless otherwise specified, extended from 9 a. m. to 4 p. m.

A large number of experiments were run at the two temperatures 20° and 11° C. This was done for the purpose of determining beyond a question of a doubt the performance of these alfalfa varieties as regards cold resistance under this particular set of conditions. Early in the work, in fact as soon as the first experiment was completed, it was found that there was a decided difference in response at the two temperatures. The difference was so striking that the results probably could have been taken as conclusive, but they were so surprising that additional data seemed necessary to substantiate them. This was done by running several more sets under much the same conditions. To avoid the possibility of exposing the plants in the short-day case to the same environmental conditions and thus reflecting the same results in the following experiments, the short-day and normal-day cases were interchanged, one being used for the short day in one experiment and for the normal day in the following experiment. This could not be done, nor did it seem essential, with the 17-hour-day case. The light from the lamp used for artificial illumination was

kept from scattering to adjacent cases by curtains which were drawn each afternoon around the 17-hour case.

The plants were kept under these special light and temperature conditions for two weeks. No attempt was made to study the time factor in connection with the influence of light on the hardening process. While it is recognized that this factor may be important, it was considered impossible adequately to carry through such a study at the time these experiments were made. The multiplicity of combinations involved in using several different varieties at different day lengths, at different temperatures, and for different lengths of time necessarily precluded as thorough a study as might have been desired. Since the 2-week period proved successful in the temperature study, and since it gave striking differences in the light study, it was considered that the underlying principle of light response might be more or less accurately determined. In all, 25 experiments were made to determine the influence of light on the hardening process. These involved the use of 1,370 porous clay pots, with a total of approximately

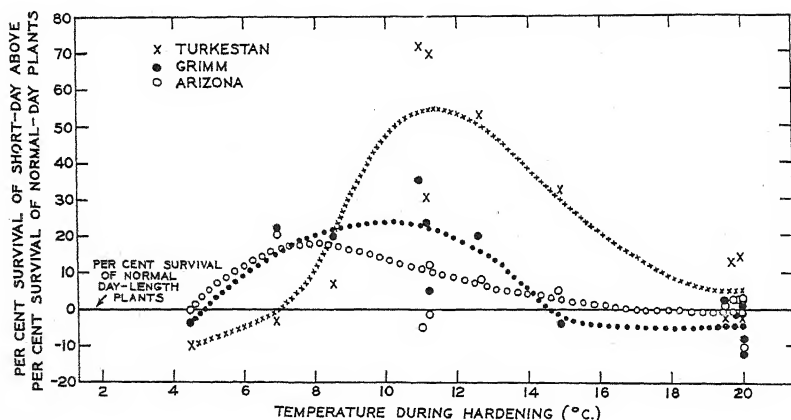


FIGURE 1.—Influence of short (7-hour) day, as compared with that of the normal day, on resistance to cold of three alfalfa varieties hardened at various temperatures

16,000 plants, assuming an average of 12 plants to the pot. The results of the uniform short-day, normal-day, and long-day experiments from which the varietal responses are calculated are given in Table 1.

In the original plan two temperatures were included, namely, approximately 10° and 20° C. The first results showed that there was a difference in response to length of day at the two temperatures. Thus, the whole question of the interrelation of light and temperature was apparently involved. Although the work has not been completed as fully as might be desired, it is believed that the results presented at this time are sufficiently consistent and include a sufficiently wide range of temperatures to indicate the principles involved.

For purposes of discussion the influence of length of day on the hardening process of the several varieties will be considered chiefly at three temperatures, viz, 20°, 11°, and below 10° C. Figure 1, based on Table 1, shows graphically the response of the three varieties to light at the different temperatures. The location of the dots is



TABLE 1.—Influence of length of day on ability of alfalfas to resist cold

Experiment No.	Hard- ening temper- ature	Freezing exposure			Repli- cations of each variety	Survival of plants frozen after 2 weeks' exposure to indicated day length												Prob- able error of the mean <sup>a</sup>	
		Length of nor- mal day during the hard- ening period	Date	Dur- ation		Tem- pera- ture	Turkistan				Grimm				Arizona common				
							7 hours	Normal day	17 hours	P. d.	P. d.	7 hours	Normal day	17 hours	P. d.	P. d.	7 hours		Normal day
21	°C. 25.0	H. m. 12 42	Apr. 8, 1931	H. m. 7 0	°C. -13.0	Number 8	P. d. 43	P. d. 97	P. d. 82	P. d. 67	P. d. 64	P. d. 76	P. d. 13	P. d. 26	P. d. 19	P. d. 17	P. d. 14	P. d. 27	P. d. 5.0
9a	20.0	10 16	Dec. 13, 1930	3 0	-14.0	5	65	67	82	64	80	76	80	31	28	15	67	4.9	5.5
2a	20.0	10 10	Nov. 19, 1929	4 15	-12.4	5	7	6	37	5	13	13	16	4	4	4	15	67	4.9
3a	19.8	9 37	Dec. 4, 1929	4 0	-12.9	5	14	0	20	0	2	2	2	3	0	2	0	2	4.9
6a	19.7	9 36	Jan. 28, 1930	3 0	-14.2	5	16	3	13	5	5	6	2	2	4	1	11	3.9	8.9
1a	19.5	10 37	Nov. 4, 1929	4 35	-12.4	5	0	2	4	2	0	0	0	1	0	0	4	0	3.8
Average							20	16	31	15	19	20	9	9	22				
15	14.8	9 56	Nov. 25, 1930	4 40	-14.6	8	49	18	44	13	17	29	19	14	19	14	19	4.8	
16	12.6	10 37	Nov. 4, 1929	4 35	-12.4	8	58	6	5	5	26	8	7	0	0	7	0	3.8	
19	12.0	9 48	Jan. 30, 1931	5 0	-13.9	8	74		32				31		10		5.4		
9b	11.2	10 16	Feb. 13, 1930	4 30	-14.2	5	88	20	49	18	13	23	44	45	49	49	5.5		
2b	11.2	10 10	Nov. 19, 1929	5 50	-13.0	5	54	24	24	27	4	13	30	18	6	18	6	4.9	
6b	11.1	9 36	Jan. 28, 1930	5 0	-14.1	5	71	0	35	27	4	12	13	9	4	9	4	3.9	
3b	10.9	9 37	Dec. 4, 1929	5 30	-13.0	5	71	0	35	35	5	5	9	0	5	5	5	4.9	
Average							71	15	36	27	5	13	14	9	5				
14	8.5	13 45	May 3, 1930	6 30	-14.7	13	81	74		45	25							5.8	
20	6.9	10 27	Feb. 17, 1931	4 45	-13.0	8	71	74	67	69	47	54	46	25	29			7.1	
8	4.5	10 15	Feb. 12, 1930	5 0	-14.2	5	84	94		87	91		54	54				5.8	
22	4.3	13 42	May 5, 1931	6 40	-13.3	16	94	76					66	50				5.7	

<sup>a</sup> Calculated by the deviation-from-the-mean method (6a).<sup>b</sup> Not included in averages.

determined for each variety by the amount by which that variety under the 7-hour day exceeds or falls short of the percentage survival of the normal-day plants at the same temperature. The normal-day plants hereafter will be referred to as checks. It should be remembered that the plants were handled similarly throughout each experiment except in the matter of variation in length of day.

At 20° C. none of the varieties under the short day differed greatly in percentage of survival from the checks grown under normal day. In no instance was the deviation from normal-day survival greater than 15 per cent. As compared to the check, Turkestan averaged slightly higher, Grimm about the same, and Arizona common slightly less. At 11°, however, the results were strikingly different. At this temperature Turkestan hardened decidedly more under the short-day

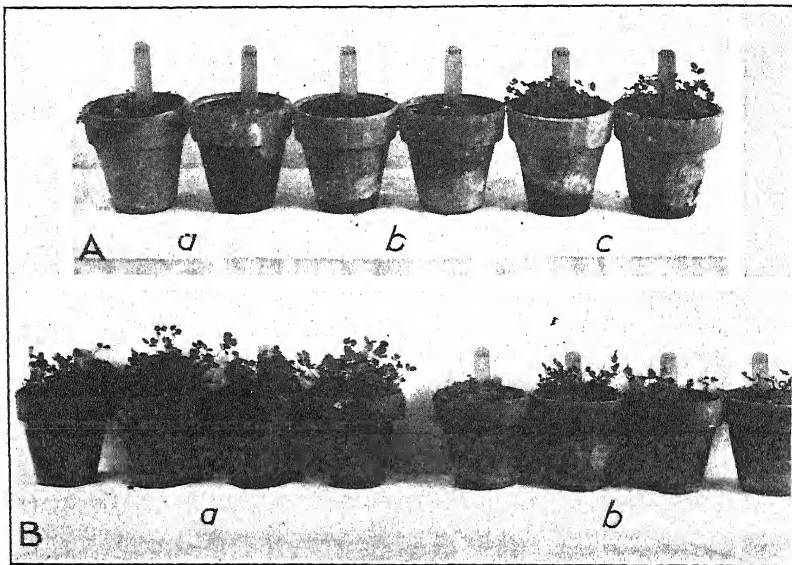


FIGURE 2.—Influence of length of day on the ability of alfalfas to resist cold: A, Plants of Turkestan variety hardened at 12° C.; a, 2 pots of plants hardened under a day length of 10 hours and 10 minutes; b, 2 pots of plants hardened under a day length of 17 hours; c, 2 pots of plants hardened under a day length of 7 hours. Photographed 14 days after freezing. B, Plants of Grimm variety hardened at 11.5°; a, 4 pots of plants hardened under a day length of 7 hours; b, 4 pots of plants hardened under a day length of 11 hours and 54 minutes. Photographed 30 days after freezing

than under the normal-day length. The average survival of the short-day plants was 71 per cent, while that of the normal-day plants was only 15 per cent. Grimm responded to the short day to a less extent than Turkestan, but considerably more than Arizona common.

Averaging the three tests conducted at approximately 11° C., in which all varieties are represented, it is found that the difference in percentage of survival in favor of the short-day plants over the normal-day plants is, for Turkestan, 56; for Grimm, 22; and for Arizona common, 5. Photographs of the plants two to four weeks after freezing are shown in Figure 2.

One experiment conducted at 25° C., when the normal length of day was 12 hours and 42 minutes, shows clearly that the 7-hour day is detrimental to the plants at this temperature and that they are not able to survive cold as well as the normal-day plants. (Table 1.)

This was the only experiment in which the short-day plants showed even a slight yellowing.

At temperatures between 10° and 20° C. the response of the varieties is found to be more or less intermediate. Turkestan apparently begins the hardening process in response to short day at a higher temperature and to a greater extent than the other two varieties. At temperatures below 10° the influence of light apparently diminishes rapidly, and at 4° there is practically no difference in survival between the short-day and the normal-day plants of any of the varieties.

If the normal-day plants are compared with the long-day plants it is found that on the average the percentage of survival is slightly higher in the long-day plants both at 10° and at 20° C. Again the response is greater in Turkestan than in either of the other varieties. At lower temperatures the long-day plants have practically the same survival as the normal-day plants in all varieties.

The increased survival of the long-day plants at the higher temperature may be explained in part by the greater growth that took place under these conditions. It has been shown that increased size, within certain limits, enables a plant better to withstand cold. The increased growth of plants under the long day is shown in Table 2, and also in Figure 3. Turkestan again is more responsive to the influence of length of day than either of the other varieties.

The Ladak variety was used in several of the later experiments because it was known that this variety begins dormancy very early in the fall. In these tests short day length influenced the hardening process in Ladak slightly more than in Turkestan, indicating a close correlation between these results and field behavior.

In three of the length-of-day experiments carried out at 11.5° and at 20° C. the height of the plants was measured just before they were put in the hardening chamber and again just before they were frozen. The actual change in the height of the plants during this 2-week period is shown in Table 2. At the lower temperature and short day, Turkestan grew 0.2 cm in two weeks and Arizona 1.4 cm. The growth of Grimm was intermediate. At the higher temperature and short day, Turkestan grew 1.9 cm and Arizona 3.2 cm. However, with a long day Turkestan grew almost as much as Arizona at both the lower and higher temperatures. These results agree with those of Oakley and Westover (16).

TABLE 2.—Growth of plants for a 2-week period under controlled conditions of light and temperature

[Average of three experiments]

Length of day	Temperature	Growth of plants during the 2-week period			Length of day	Temperature	Growth of plants during the 2-week period		
		Turkestan	Grimm	Arizona common			Turkestan	Grimm	Arizona common
<i>H. m.</i>	<i>°C.</i>	<i>Cm</i>	<i>Cm</i>	<i>Cm</i>	<i>H. m.</i>	<i>°C.</i>	<i>Cm</i>	<i>Cm</i>	<i>Cm</i>
7 0	20	1.9	1.9	3.2	7 0	11.5	0.2	0.7	1.4
10 30	20	4.3	3.9	5.3	10 30	11.5	1.5	1.8	2.3
17 0	20	5.4	4.5	6.1	17 0	11.5	3.1	3.2	3.8

The growth changes at 11° C. are in close agreement with the actual survival tests from freezing; the fact that this can not be said of the results at 20° indicates that slowing up of growth does not necessarily mean hardening of the plant.

In view of the decided hardening response of plants to the 7-hour day, further information was desired on the influence of a still shorter day, and on the length of day at which plants become weakened from lack of light. Accordingly, in December, 1929, experiments were made in which a 3-hour and a 6-hour day were used. After the

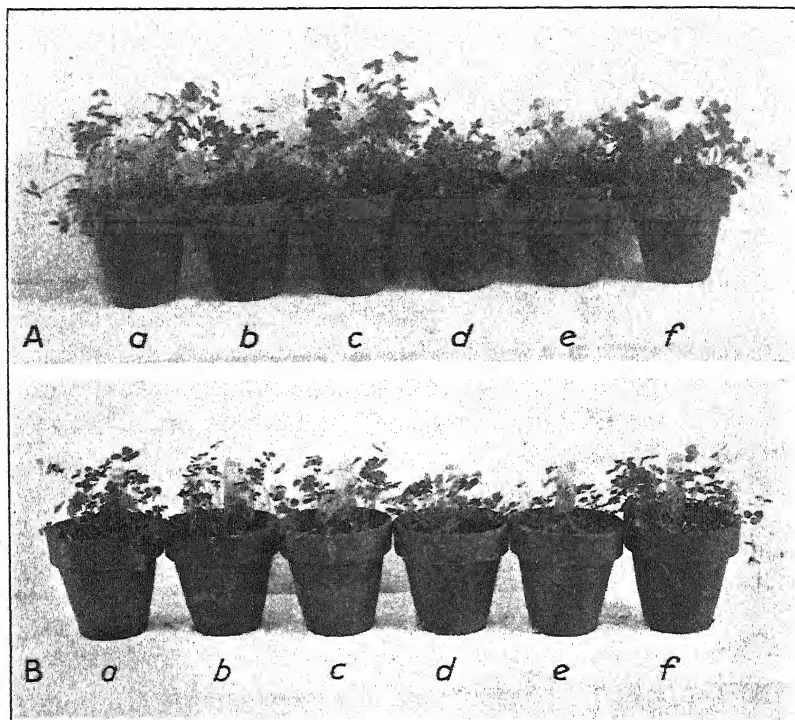


FIGURE 3.—Alfalfa varieties hardened under different day lengths and grown at different temperatures; photographed after 11 days under specified conditions and before freezing. A, Plants hardened at 20° C.: a, b, and c, Turkestan, Grimm, and Arizona varieties, under 17-hour day; d, e, and f, same varieties, under 7-hour day. B, Plants hardened at 12°: a, b, and c, Turkestan, Grimm, and Arizona varieties, under 17-hour day; d, e, and f, same varieties, under 7-hour day. Note greater growth at higher temperature and under longer day. Under short day Arizona produces more growth than either Turkestan or Grimm

plants had been kept under these short-day conditions they were frozen in the artificial freezing chamber and counts made of those that survived.

The results (Table 3) indicate that the 3-hour day was very detrimental to the plants. Even among the 6-hour-day plants the number surviving was smaller than among the normal-day plants. Experiments conducted later in the year (March) indicated that a 5-hour day might not be too short for optimum survival.

## INTERRELATION BETWEEN INFLUENCE OF LIGHT INTENSITY AND OF DAY LENGTH

During the course of the experiments on the influence of length of day on the hardening process, tests including the 7-hour day were made throughout the winter of 1930-31. It was surprising to find that during certain portions of the year even the 7-hour day did not give the high percentage of survival that it ordinarily did. The results obtained in all these studies seem to point to the fact that intensity of light as well as length of day has an important bearing on the problem. For purposes of comparison most of the experiments carried out during the period of low light intensity in the winter, together with the special experiments involving shorter day lengths, are brought together in Table 3.

TABLE 3.—*Interrelations between the influence of light intensity and that of day length on the ability of alfalfas to resist cold*

Experiment No.	Date of freezing	Total solar radiation (average per day)		Length of day	Hardening temperature	Freezing exposure		Replications of each variety	Survival				Probable error of the mean
		Outside	In hardening room			Duration	Temperature		Turkestan	Grimm	Arizona common	Ladak	
		Gram calories per cm <sup>2</sup>	Gram calories per cm <sup>2</sup>	H. m.	° C.	H. m.	° C.	Number	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
17.-----	Dec. 26, 1930	140.5 145.3	49.9 51.6	7 00 9 15	7.5 6.9	7 35 7 35	-14.1 -14.1	8 8	0 13	0 0	0 0	0 0	3.2
16.-----	Dec. 12, 1930	138.9 149.3	49.3 53.0	7 0 9 24	12.0 11.9	6 0 6 0	-14.6 -14.6	8 8	35 23	17 6	14 0	32 4	5.8
16b.-----	do.-----	138.9 149.3	49.3 53.0	7 0 9 24	14.8 14.9	5 0 5 0	-14.0 -14.0	8 8	23 27	18 40	13 8	26 5	5.8
4.-----	Jan. 4, 1930	73.2 169.9 197.6	26.0 60.3 70.1	3 0 6 0 9 15	2.9 3.0 3.0	5 0 5 0 5 0	-13.6 -13.6 -13.6	6 6 6	5 34 40	12 26 52	0 2 30	0 4 0	6.2
18.-----	Jan. 15, 1931	183.2 197.8	65.0 70.2	7 0 9 27	10.8 10.5	5 35 5 35	-13.2 -13.2	16 16	65 38	-----	9 2	-----	3.1
8.-----	Feb. 12, 1930	164.0 220.2 242.4	58.2 78.2 86.1	5 0 7 0 10 15	4.6 4.4 4.6	5 0 5 0 5 0	-14.2 -14.2 -14.2	5 5 5	94 84 94	64 87 91	51 54 54	-----	5.8
5.-----	Jan. 27, 1930	92.6 193.2 245.1	32.9 68.6 87.0	3 0 6 0 9 36	4.4 4.2 4.6	4 15 4 15 4 15	-14.5 -14.5 -14.5	5 5 5	23 64 76	39 57 70	36 54 41	-----	7.2
14.-----	May 3, 1930	208.9 281.5 375.8	74.2 99.9 133.4	5 0 7 0 13 45	8.5 8.0 8.9	6 30 6 30 6 30	-14.7 -14.7 -14.7	13 13 13	82 81 74	44 45 25	-----	-----	5.8
11.-----	Mar. 22, 1930	252.8 337.2 391.4	89.7 119.7 138.9	5 0 7 0 11 54	11.8 11.5 11.4	6 30 6 30 6 30	-14.5 -14.5 -14.5	8 8 8	97 85 76	73 85 54	26 28 27	-----	6.8

Since no apparatus for measuring light in the greenhouse was available, the radiation intensities were obtained from continuous records kept at this station by the United States Weather Bureau, by averaging the total for the given number of hours per day for the period the plants were under the special conditions. For example, if the plants were under a 5-hour day, the total radiation for those 5 hours is given. As the observations were taken on sun time they had to be corrected to standard time. A further correction was necessary because of the fact that the observations were taken in the open air,

whereas the plants were grown under four thicknesses of glass. This correction was made by the use of a correction factor worked out by placing four thicknesses of the type of glass used in the greenhouse over the pyrheliometer. Measurements with the pyrheliometer indicated that four thicknesses of glass cut down the total solar radiation to 37.9 per cent of normal when the glass was perpendicular to the rays of the sun, and to 33.2 per cent when the glass was at an angle of  $45^\circ$ . Having an air space between the glasses did not appreciably affect the reduction in solar radiation. Since the angle of the sun varies, an average figure of 35.55 was taken, and in Table 3 the solar radiation in the hardening room is calculated as 35.55 per cent that of the radiation outside. Thus, while the figures on light intensity given in Tables 3 and 4 were not taken in the greenhouse where the plants were growing, they should be approximately correct.

In Table 3 at least two, and usually three, lengths of day are given for each experiment. In all experiments the percentage survival of the plants under normal day length is used as the standard of comparison.

A summary of Table 3 is presented in Table 4, where the survivals are arranged in the order of intensity of light.

The percentage survival of the short-day plants is compared with the percentage survival of the normal-day plants, grown and frozen under the same conditions. In analyzing the results it is necessary to consider the different temperatures in the hardening chamber separately. The two main temperatures involved are  $3^\circ$  to  $4.5^\circ$  and about  $11^\circ$  C.

At  $4^\circ$  the normal-day plants are higher in survival in all experiments than the short-day plants regardless of the light intensity, but there is a greater difference in the spread at the lower intensities than at the higher. Judging by the low percentage of survival, the 3-hour day, having a total solar radiation in the hardening chamber of 26 to 32.9 gram calories per square centimeter, is obviously too short or gives insufficient light. At higher intensities and longer day, the survival of the short-day plants approaches that of the normal-day plants.

Experiment 14 of Table 3 shows that a 5-hour day may be sufficiently long if the light intensity is great enough.

At  $11^\circ$  C. the average survival, without exception, is in favor of the short day, in accordance with the decided response at this temperature, and this in spite of the fact that the light intensity is very low for some of the experiments. It should be noted that no 3-hour days were tested at this temperature, the shortest day being five hours.

The relationship between solar-radiation intensity and daylight illumination has been worked out by Kimball (9), who found a factor of 6,700 for the ratio

$$\frac{\text{Illumination intensity (foot-candles)}}{\text{Radiation intensity (gram calories per minute per cm}^2\text{)}}.$$

He states that this will give the daylight intensity within about  $\pm 5$  per cent. To use this formula on the radiation data given in Tables 3 and 4 it is necessary to divide the gram calories per square centimeter per day by the number of minutes for that particular length of day.

TABLE 4.—Summary of interrelations between the influence of light intensity and that of day length on the ability of alfalfas to resist cold

Total solar radiation (average per day)		Hardening temperature	Length of short day	Average survival of three varieties under <sup>a</sup> —		Total solar radiation (average per day)		Hardening temperature	Length of short day	Average survival of three varieties under <sup>a</sup> —	
Out-side	In hardening room			Short day	Normal day <sup>b</sup>	Out-side	In hardening room			Short day	Normal day <sup>b</sup>
<i>G cal. per cm<sup>2</sup></i>	<i>G cal. per cm<sup>2</sup></i>	<i>°C.</i>	<i>I hrs.</i>	<i>Per cent</i>	<i>Per cent</i>	<i>G cal. per cm<sup>2</sup></i>	<i>G cal. per cm<sup>2</sup></i>	<i>°C.</i>	<i>I hrs.</i>	<i>Per cent</i>	<i>Per cent</i>
73.2	26.0	3.0	3	5.7	40.7	208.9	74.2	8.5	5	<sup>d</sup> 63.0	<sup>d</sup> 49.5
92.6	32.9	4.4	3	32.7	62.3	281.5	99.9	8.5	7	<sup>d</sup> 63.0	<sup>d</sup> 49.5
164.0	58.2	4.5	5	69.7	79.7	138.9	49.3	11.8	7	22.0	9.7
169.9	60.3	3.0	6	20.7	40.7	183.2	65.0	10.6	7	<sup>e</sup> 37.0	<sup>e</sup> 20.0
193.2	68.6	4.4	6	58.3	62.3	252.8	89.7	11.6	5	65.3	52.3
220.2	78.2	4.5	7	75.0	79.7	337.2	119.7	11.6	7	66.0	52.3
140.5	49.9	7.1	7	0	<sup>c</sup> 13.0	138.9	49.3	14.8	7	18.0	25.0

<sup>a</sup> Unless otherwise shown, averages are for three varieties—Turkestan, Grimm, and Arizona.

<sup>b</sup> Normal day ranged from 9¼ hours to 13¾ hours, the total solar radiation of course being greater than that shown for the short day in the first 2 columns.

<sup>c</sup> 1 variety, Turkestan.

<sup>d</sup> 2 varieties, Turkestan and Grimm.

<sup>e</sup> 2 varieties, Turkestan and Arizona.

#### INFLUENCE OF WAVE LENGTH OF LIGHT

To determine the influence of the wave length of light on the hardening process, three cages 15 inches high, 24 inches wide, and 36 inches long were constructed of transparent cellophane. The top and sides were covered with the material, but the bottom was left open, thus allowing free circulation of air when the cages were placed on a slat bench. One cage was red; another white; and the third, blue. They were placed over the pots containing the plants in the hardening room at a temperature of approximately 3.5° C. Spectrophotometric measurements of the transmissibility of radiation by the three colors of cellophane used are given in Figure 4. The transmissibility of radiation by the clear cellophane was high at all wave lengths measured, from the blue to the red. Unfortunately, measurements could not be made of wave lengths lower than 480  $m\mu$ . The transmissibility of radiation by red cellophane was very low in the blue and the green, but increased rapidly in the yellow, the orange, and the red. The blue cellophane transmitted a high percentage of the blue, lower percentages of the green, and very little of the yellow, orange, or red. In general, therefore, the plants under the red cellophane received light from the red end of the spectrum, the plants under the blue cellophane received light from the blue end of the spectrum, and those under the clear cellophane received white light. The last mentioned was included to serve as a check.

Two separate series were run under these cages, with three different varieties, Turkestan, Grimm, and Arizona common. The results of the two series combined are presented in Table 5. In both series the plants under the white cage proved to be the most resistant to artificial freezing. The red and blue lights produced practically the same degree of hardening, the red averaging slightly less.

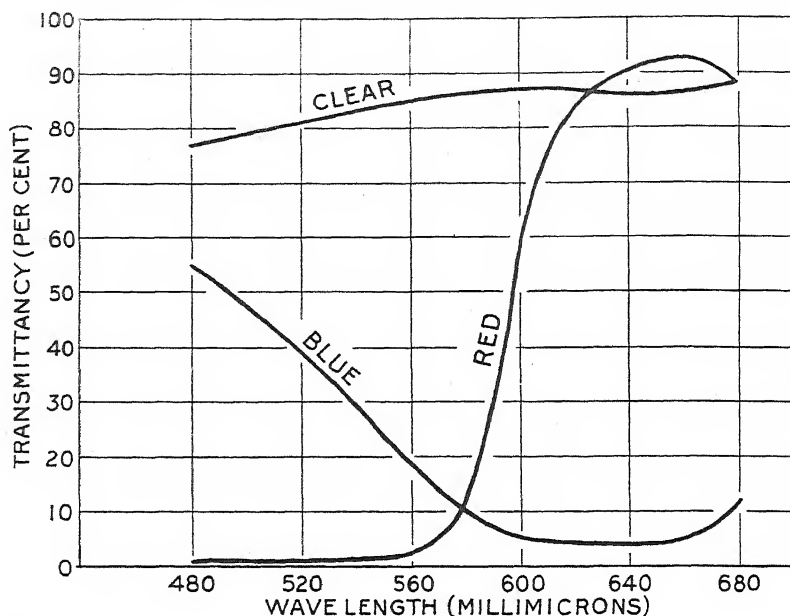


FIGURE 4.—Transmissibility of radiation by the three types of cellophane used in experiments to determine the influence of different wave lengths of light on the hardening process

TABLE 5.—Influence of light of different wave lengths on ability of alfalfas to resist cold

Light	Survival			Average of all varieties
	Turkestan	Grimm	Arizona common	
Red.....	Per cent 63	Per cent 68	Per cent 32	Per cent 54
Blue.....	60	65	50	58
White.....	77	81	44	67

## INFLUENCE OF TEMPERATURE ON THE HARDENING PROCESS

It has generally been conceded that temperature is one of the major factors in the hardening process, and hardening is usually associated with lower temperatures. However, it has been known for some time that plants can be hardened by other means than by lowering the temperature. Rosa (22) hardened cabbage plants chiefly by four methods: (1) Exposing them in an open frame to temperatures near freezing during the night and to full sunlight during the day; (2) giving them just enough water to keep them growing slowly (this lot usually showed the maximum degree of hardening in the cold frame); (3) pruning the roots severely by running a knife close to the stem on one or both sides of the plant (this treatment checked the growth of the plants materially for a short time and somewhat increased their resistance to cold); (4) by watering with N/10 solutions of various salts.

In Rosa's work (22) the degree of hardening was determined by the ability of the plants to withstand cold. He states that the growth of



plants subjected to any of the hardening treatments was checked in proportion to the intensity of the treatment and that any treatment that materially checks the growth of plants increases their resistance to cold. He used cabbage chiefly in his work, and his freezing exposures were necessarily not severe.

Harvey (6) has shown a correlation between hardening temperature and cold resistance. He states that the "threshold value" for the hardening process is approximately  $5^{\circ}$  C. for cabbages. He also suggests that alternating temperatures harden cabbages and that 1 hour's exposure to cold in every 24 hours has a hardening effect.

While the importance of temperature in the hardening process is more or less taken for granted, very few actual data except those confined to horticultural crops can be found which show the relation of hardening temperatures to ability to withstand cold. Having this in mind, and also the ever-present problem of causing as wide differences between hardy and nonhardy varieties as possible, the writer made several experiments under controlled temperature conditions, to determine the relation of hardening temperatures to cold resistance.

#### INFLUENCE OF VARIOUS CONSTANT TEMPERATURES

The cases in the hardening room could be held at any desired temperature above that of the hardening room with a variability of about  $1^{\circ}$  C. During the season 1930-31 two cases were fitted with blowers which drew cold air from around artificially refrigerated coils, discharging this air directly into the case. By this means these cases could be maintained at a relatively constant temperature below that of the hardening room and as low as  $-5^{\circ}$  C.

Altogether, 10 experiments were carried out in which temperatures ranging from  $-5^{\circ}$  to  $30^{\circ}$  C. were used. The plants remained under these special hardening conditions for 14 days. The object in using such a wide range of temperatures was to determine at what temperature hardening begins, when it reaches a maximum, and how low it may be before complete killing occurs. Other factors studied involved varietal response and the determination of the portion of the temperature scale at which hardening progresses most rapidly for each change of 1 degree in temperature.

The results of these experiments, presented in Table 6, show that within certain ranges the lower the hardening temperature the greater is the ability of the plants to resist cold. Experiments 1, 2, and 3, with temperatures ranging from  $2^{\circ}$  to  $20^{\circ}$  C., show a progressively higher percentage of survival at each progressively lower temperature. In fact, in experiments 2 and 3, the plants hardened at the higher temperatures were not exposed so long to freezing as those hardened at the lower temperatures, for if frozen at the same temperature the former would be completely killed, while the latter would show very little killing.

To determine the highest temperature at which the hardening process begins, experiments 4 and 5 were conducted. Temperatures of  $10^{\circ}$ ,  $15^{\circ}$ , and  $20^{\circ}$  C. were used. The results show that a certain amount of hardening takes place at  $15^{\circ}$ . In experiment 6, in which temperatures of  $15^{\circ}$ ,  $20^{\circ}$ , and  $25^{\circ}$  are compared, it is interesting to note that the plants hardened at  $20^{\circ}$  show a slightly greater resistance to cold than those hardened at  $25^{\circ}$ .

TABLE 6.—*Influence of temperature during hardening on the ability of alfalfas to resist cold*

Experiment No. and period <sup>a</sup>	Hardening temperature	Freezing exposure		Replication in each variety	Survival of—						Probable error of mean
		Duration	Temperature		Turkestan	Grimm	Ari-zona common	Ne-bras-ka common	Utah common	La-dak	
	°C	H. m.	°C	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1, Oct. 2 to Nov. 4, 1929.....	7.1	4 35	-12.4	6	96	75	41	98	71	-----	4.7
	8.0	4 35	-12.4	6	96	48	22	85	63	-----	4.7
	10.5	4 35	-12.4	6	60	20	3	24	11	-----	4.7
	12.3	4 35	-12.4	6	20	6	0	-----	-----	-----	4.7
	19.5	4 35	-12.4	6	4	0	0	-----	-----	-----	4.7
2, Oct. 8 to Nov. 19, 1929.....	3.6	5 50	-13.0	5	98	98	96	96	98	-----	4.3
	5.3	5 50	-13.0	5	93	97	61	92	89	-----	4.3
	9.1	5 50	-13.0	5	79	53	26	16	49	-----	4.3
	11.3	5 50	-13.0	5	18	13	18	6	20	-----	4.3
	19.9	4 15	-13.0	5	6	13	15	8	5	-----	4.3
3, Oct. 24 to Dec. 4, 1929.....	2.0	7 25	-13.0	5	77	59	34	37	75	-----	6.8
	4.7	7 25	-13.0	5	62	23	10	29	21	-----	6.8
	8.6	5 30	-13.0	5	18	7	0	1	0	-----	6.8
	10.9	5 30	-13.0	5	0	0	0	0	0	-----	6.8
	19.7	4 0	-13.0	5	0	0	0	0	0	-----	6.8
4, Oct. 10 to Nov. 25, 1930....	9.9	3 30	-13.1	8	73	83	58	-----	-----	86	5.2
	14.3	3 30	-13.1	8	44	56	33	-----	-----	38	5.2
	20.2	3 30	-13.1	8	10	11	11	-----	-----	15	5.2
5, Oct. 27, to Dec. 11, 1930....	10.3	4 0	-12.0	8	80	75	46	-----	-----	70	4.8
	14.9	4 0	-12.0	8	38	36	12	-----	-----	19	4.8
	20.0	4 0	-12.0	8	18	19	5	-----	-----	3	4.8
6, Nov. 11 to Dec. 27, 1930....	15.0	3 30	-9.1	8	67	52	25	-----	-----	48	4.7
	20.4	3 30	-9.1	8	31	12	4	-----	-----	14	4.7
	24.5	3 30	-9.1	8	18	6	0	-----	-----	12	4.7
7, Dec. 12, 1930, to Jan. 31, 1931.....	5.6	5 0	-13.6	16	89	-----	53	-----	-----	-----	13.2
	10.2	5 0	-13.6	16	80	-----	51	-----	-----	-----	13.2
	-2.5	5 0	-13.6	16	0	-----	0	-----	-----	-----	13.2
8, Dec. 31, 1930, to Feb. 16, 1931.....	20.2	2 0	-12.8	8	38	24	13	-----	-----	11	5.2
	24.1	2 0	-12.8	8	32	20	16	-----	-----	13	5.2
	29.9	2 0	-12.8	8	55	21	44	-----	-----	17	5.2
9, Sept. 9 to Oct. 22, 1931....	0	8 45	-13.4	10	43	21	12	-----	-----	-----	5.5
	6.0	8 45	-13.4	10	68	25	0	-----	-----	-----	5.5
10, Sept. 19, to Nov. 7, 1931....	0	5 45	-13.7	10	55	53	22	-----	-----	-----	4.4
	6.0	5 45	-13.7	10	80	84	61	-----	-----	-----	4.4

<sup>a</sup> Date of seeding to date of freezing.

The influence of hardening temperatures below 0° C. is shown in experiment 7. It was intended to keep the temperature in one of the experimental cases at -5°, but owing to the large number of warm, sunny days the average temperature for the 2-week period was -2.5°. The temperature rarely, if ever, rose above 0°. In this experiment only half of the pots were exposed to the freezing temperature shown in Table 6. The other half were taken directly from the hardening room to the warm greenhouse without exposure in the freezing room. None of these plants recovered. Thus it is clear that plants taken from a warm greenhouse and exposed directly to a temperature as low as -5° will not survive.

In experiment 8 the hardening temperatures were higher than in any of the other experiments; that is, 20°, 24°, and 30° C. The percentage survival at 20° is higher than at 24°, and the percentage survival at 30° is also higher than at 24°. Figure 5 shows the recovery of alfalfas exposed to freezing after being hardened at various temperatures.

The influence of a constant temperature very near 0° C. on the hardening process is shown in experiments 9 and 10. When the

average of both experiments is considered, for all three of the varieties tested, it is evident that plants hardened at  $0^{\circ}$  are not so resistant to cold as those hardened at  $6^{\circ}$ .

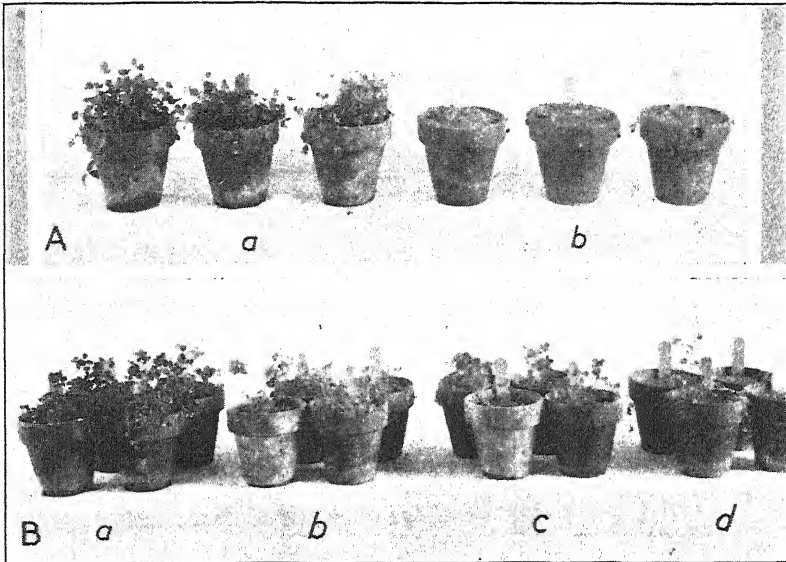


FIGURE 5.—Influence of temperature on the ability of alfalfas to resist cold: A, Grimm variety; a, 3 pots in hardening chamber at  $4^{\circ}$  C. two weeks before freezing; b, 3 pots not in hardening chamber before freezing. B, Turkestan variety, hardened for 14 days at various temperatures; a, 4 pots at  $4^{\circ}$ ; b, 4 pots at  $10^{\circ}$ ; c, 4 pots at  $14^{\circ}$ ; d, 4 pots at  $20^{\circ}$ . All plants frozen for same length of time in artificial freezing chamber

Since the experiments were not conducted at the same time and since the freezing temperatures were necessarily different in different experiments, it is difficult to form a composite picture of the results

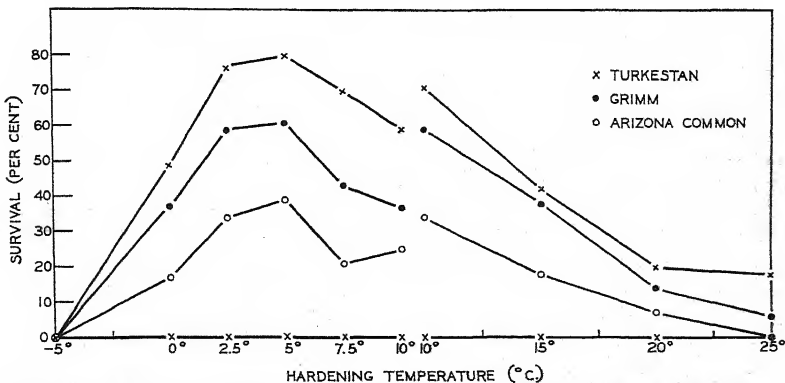


FIGURE 6.—Influence of hardening at different temperatures on the ability of three alfalfa varieties to resist cold

of the entire temperature range. Nevertheless, in Figure 6, an attempt has been made to combine the results of all experiments. The average percentage survival at each of the temperatures shown on the base line has been calculated from Table 6. The temperatures indicated

on the base line are taken as mid-points, and the average of all experiments with hardening temperatures nearest are included at these points. For example, all experiments having a hardening temperature between  $12.5^{\circ}$  and  $17.5^{\circ}$  are included in the average at  $15^{\circ}$  C. The figure has been divided into two parts, above  $10^{\circ}$  and below  $10^{\circ}$ , because those plants hardened at temperatures below  $10^{\circ}$  were as a rule frozen more severely than those hardened above  $10^{\circ}$ .

Figure 6 shows that plants hardened at  $20^{\circ}$  C. have a slightly higher percentage survival than those hardened at  $25^{\circ}$ . This difference, however, is not so marked as that between  $15^{\circ}$  and  $20^{\circ}$  or between  $10^{\circ}$  and  $15^{\circ}$ . The two hardy alfalfas, Turkestan and Grimm, show greater hardening at these temperatures than does the nonhardy Arizona common. The resistance of these varieties to cold increases when they are hardened at lower temperatures, maximum resistance being reached between  $2.5^{\circ}$  and  $5^{\circ}$ . When the plants are hardened at a constant temperature of  $0^{\circ}$  they show less hardiness than when hardened at  $5^{\circ}$ . No plants of any variety exposed to a temperature of  $-5^{\circ}$  for a period of two weeks survived. If temperatures between  $-5^{\circ}$  and  $0^{\circ}$  had been used it might have been found that all varieties were not killed at the same temperature.

Figure 6 shows that within certain limits there is a gradual increase in hardening as temperatures are lowered. These limits spread over a relatively wide range, from approximately  $20^{\circ}$  to near  $0^{\circ}$  C. There is no definite point at which hardening does or does not occur, the hardening process apparently being a gradual development. The amount of hardening for  $1^{\circ}$  change in temperature between  $15^{\circ}$  and  $20^{\circ}$  is not nearly so great as between  $5^{\circ}$  and  $10^{\circ}$ . The maximum rate of hardening for 1 degree change in temperature occurs at about  $5^{\circ}$ . Hardening of tender alfalfa plants at continuous temperatures much below  $0^{\circ}$  can not occur, for the plants are killed outright. Turkestan is more responsive to a change in temperature than Grimm, and Grimm than Arizona common. (Fig. 6.) This is directly correlated with the hardiness of the three varieties.

#### INFLUENCE OF ALTERNATING TEMPERATURES

In all the experiments thus far reported temperature and light were kept as uniform as possible. In the experiments now to be reported the hardening temperature was held constant for one set of plants, while duplicate sets were moved from one temperature chamber to another at intervals of 3 days. All plants remained in the hardening room for 12 days, and the set that was rotated went through four temperature changes. Thus, one set remained at  $20^{\circ}$  C. throughout the 12 days. A duplicate set was started at  $15.4^{\circ}$ , where it remained for 3 days; transferred to  $11.4^{\circ}$ , where it remained for 3 days; then to  $5^{\circ}$  for 3 days; and finally to  $20^{\circ}$  for 3 days, from which it was taken directly to the freezing chamber. In this experiment only one variety, Grimm, was used.

The results of two experiments have been combined and are presented in Table 7.

Table 7 shows that at  $20^{\circ}$  and  $19.5^{\circ}$  C. the percentage survival of plants from the alternating temperature was greater than that of plants from the continuous high temperature. Since the alternating-temperature plants had been exposed to a much lower temperature during the preceding three days, the increased survival may be attrib-

uted to a carry-over effect from the lower temperature. That this effect at the end of three days was not very great may be seen from the small differences recorded.

TABLE 7.—*Influence of alternating temperatures on the ability of Grimm alfalfa to resist cold*

Hardening temperatures (°C.) <sup>a</sup>	Percent- age sur- vival <sup>b</sup>	Hardening temperatures (°C.) <sup>a</sup>	Percent- age sur- vival <sup>b</sup>
15.4 to 11.4 to 5 to 20 .....	2	5 to 20 to 15.4 to 11.4 .....	29
20 continuous .....	0	11.4 continuous .....	34
14.3 to 10.8 to 3.5 to 19.5 .....	14	3.5 to 19.5 to 14.3 to 10.8 .....	14
19.5 continuous .....	10	10.8 continuous .....	62
11.4 to 5 to 20 to 15.4 .....	11	20 to 15.4 to 11.4 to 5 .....	0
15.4 continuous .....	0	5 continuous .....	19
10.8 to 3.5 to 19.5 to 14.3 .....	6	19.5 to 14.3 to 10.8 to 3.5 .....	0
14.3 continuous .....	24	3.5 continuous .....	14

<sup>a</sup> 3 days at each temperature.

<sup>b</sup> After freezing 7 hours at  $-15^{\circ}$  C.

At  $15.4^{\circ}$  C. the behavior of the plants was much the same as at  $20^{\circ}$ , but at  $14.3^{\circ}$  a greater degree of hardening occurred than in those plants that passed through three days at higher temperatures. Moreover, from  $11.4^{\circ}$  to  $3.5^{\circ}$  the plants under the continuous temperature survived better than those that had been subjected to the high as well as the low temperatures, showing that a 3-day period at constant temperature did not harden the plants to the same degree as 12 days under the same conditions. Bearing in mind the influence of hardening temperatures on survival, it is interesting to note that increased survival of the plants under continuous treatment began at approximately  $14^{\circ}$ .

In order to study alternating temperatures on the basis of a 24-hour day, further experiments were conducted with the Turkestan and Arizona common varieties. In experiment 3, the results of which are given in Table 8, 16 pots of each variety were placed in a case held at an average temperature of  $0.6^{\circ}$  C. Each day eight pots of each variety were removed to a warm greenhouse (approximately  $20^{\circ}$ ) and kept there for 8 hours (8 a. m. to 4 p. m.). The remainder of the pots were kept in the case. On the morning of the fifteenth day all the pots were transferred directly from the case to a freezing temperature of  $-13.2^{\circ}$ , at which they were held for  $5\frac{1}{2}$  hours. The resulting survivals show that the plants subjected to alternating temperatures were able to resist the cold much more effectively than those held continuously at a low temperature.

A preliminary hypothesis, based on the results of experiment 3, assumed that the increase in hardness of the alternating-temperature plants was due to the fact that at a high temperature the physicochemical processes stimulated by the low temperature could be carried on much more rapidly than in plants remaining at a temperature near  $0^{\circ}$  C. If this hypothesis is correct, alternating temperatures above a certain temperature should not give increased hardness. This temperature would be the point at which the physicochemical processes could be carried on at as rapid a rate as the stimulus warranted, and no further increase in the rate of this reaction could be obtained by means of higher temperatures.

To test this hypothesis experiment 4 was conducted. Five different temperatures ranging from  $-5^{\circ}$  to  $11.3^{\circ}$  C. were used. As in experiment 3, half of the plants were allowed to remain eight hours during daylight in the warm greenhouse. The results (Table 8) support the hypothesis.

TABLE 8.—*Influence of alternating temperatures on the ability of alfalfas to resist cold*

Experiment No. and period *	Hard- ening tem- pera- ture	Freezing expo- sure		Rep- lica- tions of each vari- ety	Survival after indicated condi- tions during hardening				Prob- able error of mean	
		Dura- tion	Tem- pera- ture		Turkestan		Arizona com- mon			
					Con- stant tem- pera- ture	In warm green- house 8 hours each day	Con- stant tem- pera- ture	In warm green- house 8 hours each day		
	$^{\circ}$ C.	H.	m.	$^{\circ}$ C.	Num- ber	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
3, Dec. 31, 1930, to Feb. 17, 1931...	0.6	5	30	-13.2	8	44	87	8	87	6.9
4, Jan. 17 to Mar. 3, 1931.....	1.6	5	0	-13.6	8	40	74	14	77	6.9
	4.2	5	0	-13.6	8	69	88	79	75	6.9
	7.7	4	30	-13.2	8	92	92	76	55	6.9
	11.3	4	30	-13.2	8	88	76	47	51	6.9
5, Feb. 4 to Mar. 20, 1931.....	7.8	6	0	-13.0	8	84	98	72	65	5.6
	11.8	6	0	-13.0	8	77	39	36	49	5.6

\* Date of seeding to date of freezing.

The  $-5^{\circ}$  C. set is omitted from the table because at that temperature all the plants, even the alternating-temperature ones, were killed outright.

At a temperature of  $1.6^{\circ}$  C. the alternating-temperature plants were considerably higher in survival in both varieties. At  $4.2^{\circ}$  the Turkestan variety had a higher percentage survival at the alternating temperature, whereas Arizona common had a slightly lower survival. It is clear from these and later results that the hardy variety Turkestan must be treated separately from the unhardy variety Arizona common when speaking of their hardening response, since the former has a much greater capacity for hardening than the latter.

At  $7.7^{\circ}$  C. the percentage survival of Turkestan for both the continuous and alternating temperatures was the same, and at  $11.3^{\circ}$  there was a distinct reversal in favor of the continuous temperature, indicating that subjecting the plants to a still higher temperature for eight hours each day serves actually to lessen the amount of hardening. From these results it appears that the point at which the hardening activity is not greatly increased by higher temperatures is approximately  $8^{\circ}$  for the hardy variety and somewhat lower for the unhardy variety.

The relative increase in percentage survival of Arizona common at  $11.3^{\circ}$  C. may perhaps be explained by the added growth, as distinguished from hardening, which is possible at the higher temperature. This is not true of Turkestan, for it shows much more hardening at this temperature. (Fig. 6.)

The question arises as to the part played by light in the alternating-temperature effect. Since it has been demonstrated that light is an

important factor in the hardening process, might it not be true that through its influence on photosynthesis it has the ability to counterbalance the entire process of hardening?

To study this question and also to determine the length of time required at the low temperature for optimum stimulating effect on the hardening process, an experiment was conducted in which one hardening temperature, 3.8° C., and different alternating periods were used. In this experiment, reported in Table 9, one set of eight pots of each of the two varieties was kept in the hardening room (held at 3.8°) for a certain number of hours each day and then removed to the warm greenhouse (approximately 20°). In all, five sets of plants of each variety received this treatment. A sixth set remained in the hardening room continuously. Two additional sets were placed in two cases used in experiment 5, Table 8, and held at temperatures of 8° and 12°. Half of the pots in each of these cases were removed to the warm greenhouse and kept there for eight hours each day (8 a. m. to 4 p. m.). These last two sets were used for the purpose of checking the results in experiment 5, Table 8. The results checked admirably with those shown in experiment 5, Table 8.

TABLE 9.—*Influence of various periods of alternating temperatures and their relation to light in the hardening process of alfalfas*

Length of time in hardening room each day <sup>a</sup>	Period of day in hardening room	Survival after freezing <sup>b</sup>		Length of time in hardening room each day <sup>a</sup>	Period of day in hardening room	Survival after freezing <sup>b</sup>	
		Turkestan	Arizona common			Turkestan	Arizona common
Hours		Per cent	Per cent	Hours		Per cent	Per cent
2	8 a. m. to 10 a. m. ....	77	66	16	4 p. m. to 8 a. m. ....	95	38
4	8 a. m. to 12 m. ....	90	43	24	Continuously .....	80	65
8	8 a. m. to 4 p. m. ....	75	55	8	8 a. m. to 4 p. m. ....	41	15

<sup>a</sup> The hardening room was held at 3.8° C.

<sup>b</sup> After 2 weeks of treatment, all the plants were frozen 5½ hours at -13.9° C.

<sup>c</sup> This set was taken directly from the hardening room to a dark, warm case; the other sets were exposed to normal day length.

At the end of 14 days under the conditions described above the plants were frozen in the artificial freezing room. Those that had been in the warm greenhouse during the day had a healthy dark-green color, whereas those that had been in the hardening room during the day and in the dark chamber the rest of the time were light green. The plants continuously in the hardening room were not so dark a green as those that had been eight hours in the warm greenhouse during the day. The other sets were of a uniformly dark-green color.

With reference to the results from the Turkestan variety (Table 9), it is evident that the 2-hour exposure in the hardening room was not sufficient to give optimum hardening. Especially interesting is the fact that the plants kept in the hardening room 8 hours during the day did not have so high a survival as those kept 4 or even 2 hours. These results might be explained on the basis of the short period of light remaining to the plants in the warm greenhouse. Further evidence in support of this explanation is the fact that the plants kept for 8 hours during the day in the hardening room and for the rest of the time in a warm dark room had a very much lower survival; in

fact, the lowest survival of any of the groups. Representative pots from these sets are shown in Figure 7.

Arizona common did not respond to the hardening conditions as did Turkestan (fig. 6), and the results were not so clear-cut. For instance, the survival in Arizona common after four hours' exposure in the hardening chamber was not so high as after two hours. This is contrary to the results with Turkestan. However, Arizona common and Turkestan showed a similar response with respect to the influence of the dark chamber, the plants that were taken directly to the dark room from eight hours in the hardening room being the lowest in percentage survival.

Harvey (6) reports that alternate equal exposures during hardening of 12 hours at 0° C. and 12 hours at 10° or at 20° produce greater hardness than continuous exposures at the average of these temperatures, i. e., 5° and 10°, respectively. The results obtained in alfalfa experiments indicate a greater response to alternate temperatures than was obtained in experiments with cabbage.

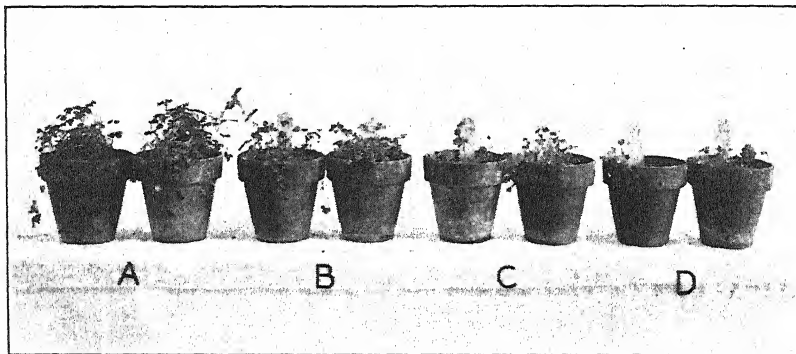


FIGURE 7.—Influence of alternating temperatures and of light on the ability of Turkestan alfalfa to resist cold: A, Two pots, 16 hours in the hardening room, 8 hours (8 a. m. to 4 p. m.) in warm greenhouse; B, 2 pots, continuously in hardening room; C, 2 pots, 8 hours (8 a. m. to 4 p. m.) in hardening room, rest of time in warm greenhouse; D, 2 pots, 8 hours (8 a. m. to 4 p. m.) in hardening room, rest of time in dark case in warm greenhouse. The average temperature of the hardening room during the 2-week period was 3.8° C. All pots were frozen for 5½ hours at -13.9°. Photographed two weeks after freezing

Table 10 gives the results of an experiment designed to show in greater detail the importance of the interrelations between light and temperature. All three sets of pots, each set containing eight pots of each variety, during a period of two weeks previous to freezing were exposed to seven hours of daylight, from 9 a. m. to 4 p. m., and for the rest of each day were kept in light-proof cases. The difference in treatment lies in the fact that each set was exposed to different conditions of temperature when exposed to light. One set when exposed to light was in the hardening room at 2° C., and the rest of the time in a dark warm case. This set showed the lack of photosynthetic activity by its yellowish-green color at the time of freezing and was decidedly the lowest in survival. A second set was kept continuously at 25°, but with only seven hours of light it was unable to resist the cold effectively, although it had a percentage of survival somewhat above that of the first set. This set was a lighter green than the third set, but not the yellowish green of the first. In contrast to these two sets, the third set, exposed to light at a temperature of 25° for seven



hours and placed in the hardening room at 2° for the rest of the time, had a very high percentage survival.

Thus alternating temperatures, with light during the warm periods, seem to furnish the optimum conditions for hardening.

TABLE 10.—*Influence of light and its relation to alternating temperatures in the hardening process of alfalfa*

Plants for 2 weeks previous to freezing kept in—	Survival after freezing <sup>a</sup>	
	Turkestan	Arizona common
	Per cent	Per cent
Light from 9 a. m. to 4 p. m. at 2° C.; dark case at 25° rest of time.....	5	2
Light from 9 a. m. to 4 p. m. at 25° C.; dark case at 25° rest of time.....	43	6
Light from 9 a. m. to 4 p. m. at 25° C.; dark case at 2° rest of time.....	91	77

<sup>a</sup> All plants were frozen 7 hours at -13.0° C.

### INFLUENCE OF MOISTURE CONTENT OF THE SOIL ON THE HARDENING PROCESS

Rosa (22) reported that drought increased the resistance of cabbage to cold practically as much as low temperatures. Maximov (16) and Newton and Martin (14) suggested a close parallellism between drought and cold resistance.

An attempt was therefore made to determine what part drought plays in the hardening process. Plants grown in water-tight metal containers in the warm greenhouse at optimum soil moisture were hardened at different soil moistures. These metal containers were 4 inches square by 6 inches deep. Two hundred and fifty were filled with 1,000 g each of dry soil having a moisture equivalent of 23.8 per cent. The five varieties, Turkestan, Grimm, Nebraska common, Utah common, and Arizona common, were allowed to grow one month in the warm greenhouse under approximately optimum soil-moisture conditions. Just before the cans were taken to the hardening room they were allowed to dry until they contained about 14 per cent moisture. This process of drying out required three days. It was noticed that many plants showed incipient wilting at this moisture content.

When the soil was sufficiently dry, one-third of the metal containers were made up, by weighing, to a moisture content equal to three-fifths moisture equivalent, or 14.3 per cent; another one-third to 1 moisture equivalent, or 23.8 per cent; and the rest to 1½ moisture equivalent, or 33.3 per cent. All were then put in the hardening room, where they remained at an average temperature of 4.9° C. for 14 days. The moisture content of the soil was checked occasionally by weighing, and was kept relatively uniform, although it was impossible to keep it at the exact figure mentioned in the text.

At the end of the 2-week period the moisture in half of the metal containers at 14.3 per cent was made up to 33.3 per cent by adding the correct amount of water. Similarly, the moisture in half of those at 23.8 per cent was made up to 33.3 per cent. As soon as this operation was completed (in about 30 minutes) the cans were placed in the freezing chamber. By the time they were put in the freezer the water had percolated into the soil. All sets were frozen for the same length

of time, seven hours at  $-11.9^{\circ}\text{C}$ . At the end of the 2-week period it was found that there was a great difference in the percentage survival of the different groups.

Since it is believed that there are no outstanding differences between the responses of the different varieties to this treatment, and since, by using the average of the varieties, a great many more individuals can be included, the discussion of results will be based on the average of all varieties given in the last column of Table 11.

TABLE 11.—*Influence of moisture content of the soil on the hardening process in alfalfas*

Experiment No. and period <sup>a</sup>	Moisture in soil		Freezing exposure			Replications each variety	Survival						
	During hardening <sup>b</sup>	During freezing	Duration	Average temperature	Minimum soil temperature		Turkestan	Grimm	Nebraska common	Utah common	Arizona common	Ladak	Average
1, Nov. 23, 1929, to Jan. 29, 1930.....	<i>P. ct.</i>	<i>P. ct.</i>	<i>H. m.</i>	$^{\circ}\text{C}$ .	$^{\circ}\text{C}$ .	<i>No.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
	14.3	14.3	7 0	-11.9	-11.2	7	75	52	20	57	37	---	48
	14.3	33.3	7 0	-11.9	-3.8	6	87	72	65	97	80	---	80
	23.8	23.8	7 0	-11.9	-5.4	7	71	86	72	76	79	---	77
	23.8	33.3	7 0	-11.9	-3.8	6	95	82	89	85	82	---	87
	33.3	33.3	7 0	-11.9	-3.8	16	76	66	70	85	73	---	74
2, Dec. 10, 1929, to Feb. 20, 1930.....	15.0	15.0	4 30	-14.2	-9.4	9	---	---	53	47	---	---	50
	15.0	30.0	4 30	-14.2	-6.1	7	84	94	---	---	19	---	66
	15.0	30.0	5 25	-14.4	-9.7	9	---	---	35	37	---	---	36
	30.0	30.0	4 30	-14.2	-6.1	8	95	74	78	65	19	---	63
	30.0	30.0	5 25	-14.4	-9.7	9	---	---	25	41	---	---	72
	30.0	30.0	5 25	-14.4	-9.7	9	---	---	---	---	---	---	33
3, Feb. 17 to Apr. 8, 1930.....	14.3	14.3	8 53	-15.6	-10.7	22	---	19	---	---	---	---	---
	14.3	35.0	8 53	-15.6	-3.8	22	---	65	---	---	---	---	---
	14.3	35.0	12 18	-16.5	---	22	---	39	---	---	---	---	---
	23.8	23.8	8 53	-15.6	-6.4	22	---	62	---	---	---	---	---
	23.8	35.0	8 53	-15.6	-3.7	22	---	72	---	---	---	---	---
	23.8	35.0	12 18	-16.5	-9.9	22	---	26	---	---	---	---	---
	35.0	35.0	8 53	-15.6	-3.9	20	---	86	---	---	---	---	---
	35.0	35.0	12 18	-16.5	-15.5	20	---	5	---	---	---	---	---
	Dry.	+13.0	3 0	-13.0	-8.2	5	8	15	---	---	36	67	32
	do.	+45.0	3 0	-13.0	-4.0	5	92	86	---	---	88	89	89
4, Oct. 27 to Dec. 12, 1930.....	( <i>f</i> )	+25.0	3 0	-13.0	-2.5	5	38	69	---	---	65	47	55
	( <i>f</i> )	+45.0	3 0	-13.0	-2.5	5	94	97	---	---	84	71	87

<sup>a</sup> Date of seeding to date of freezing.

<sup>b</sup> In experiments 1, 2, and 3 the plants were hardened at a temperature of  $1^{\circ}$  to  $-5^{\circ}\text{C}$ . during the 14-day period of special soil-moisture treatments. Experiment 4 was conducted at a room temperature of approximately  $20^{\circ}$ .

<sup>c</sup> Average of the varieties Turkestan, Grimm, and Arizona common.

<sup>d</sup> Average of the varieties Nebraska common and Utah common.

<sup>e</sup> Approximate percentage.

<sup>f</sup> Medium wet.

It may be seen from Table 11 that the plants in experiment 1, which were frozen at the low moisture content (14.3 per cent), had a much lower average percentage survival than any of the others. It may also be seen that the minimum soil temperature was much lower. No outstanding differences in percentage of survival are shown between the three sets frozen at 33.3 per cent moisture content, although they were hardened at greatly different moisture contents. In the set hardened at the high moisture content (33.3 per cent) the percentage of survival was slightly lower than in the others.

In order to determine whether the low percentage survival of the low-moisture group was due to the difference in soil temperature, experiment 2 was carried out in much the same way as experiment 1, except that at the time of freezing two sets instead of one were made

up to the high moisture content. Only two soil moistures (15 and 30 per cent) were used in this experiment.

When the freezing tests were made, one set of containers with low moisture and one set with high moisture were taken out at the same time; the other set with high moisture was allowed to remain in the freezer until the soil temperature approximated that of the low-moisture set.

Since different varieties were used in this test, a strict comparison can not be made between all groups, but a sufficient number may be made to show that those frozen at high moisture for the same period as those at low moisture were much higher in average percentage survival. On the other hand, among those frozen at the same moisture content there is a very slight difference in favor of those that were hardened at the low moisture content. However, no great significance can be attached to this difference.

Those that were frozen at a high moisture content for a longer period, until the soil temperature approached that of the low-moisture group, did not have quite so high an average percentage survival as those of the low-moisture group. The average percentage of survival among those that were frozen at the high moisture content was 36 and 33 per cent as compared with 50 per cent for those both hardened and frozen at a low moisture content. The soil temperature actually averaged slightly lower in the former group than in the latter.

In experiment 3 a somewhat larger number of cans was used, although only one variety, Grimm, was employed. As in experiment 2, two sets with high moisture were made up from the low-moisture group.

In experiment 3, there was a survival of 19, 62, and 86 per cent among the plants frozen for the same length of time with 14.3, 23.8, and 35 per cent moisture, respectively, and a survival of 65, 72, and 86 per cent for those from the low, medium, and high moisture groups, made up to high moisture content just before freezing and frozen for the same length of time as the previous group.

In the third group there was a survival of 39, 26, and 5 per cent with low, medium, and high moisture, respectively, when made up to high moisture and frozen for a long period of time. In this group there was a slight difference in favor of those hardened at the low soil-moisture content. The average of this group (23 per cent), representing the exposure calculated to give the same soil temperature as that with low moisture, corresponds closely to the 19 per cent survival for the low-moisture group. Representative plants from experiment 3, two weeks after freezing, are shown in Figure 8.

During the fall of 1930 a fourth experiment was undertaken. The plants were grown in pots and kept in a warm room at approximately 20° C. during the hardening period. A check was not kept on the percentage of moisture in the soil, but the pots of each variety were divided into two groups, one of which was watered normally and the other maintained as closely as possible at the incipient wilting point of the plants. This point could easily be determined by the slight wilting of the leaves which occurred in all plants of this group. At the end of two weeks of this treatment the plants were frozen, necessarily very gently, in the artificial freezing chamber. Immediately before freezing, half of the pots of each group were saturated with water,

but before the plants were completely frozen the excess water had had a chance to drain off.

The results in Table 11 show that decidedly greater killing occurred among the plants with low moisture content. The plants in the medium-wet soil survived considerably better, but not so well as those in the wet soil. There was very little difference between those hardened by drying and those watered normally, when frozen with the same moisture content; this difference was in favor of the low-moisture plants.

A very interesting point in connection with this experiment is that after the freezing there were some plants that had not been injured, the leaves remaining turgid. A careful tabulation of these showed 1 pot of Turkestan, 3 of Grimm, 3 of Arizona common, and 6 of Ladak. All of these were from the group that had been hardened by maintaining a low moisture content. However, even though the tops of some of the plants appeared uninjured, the average percentage survival for all the plants in this group was much lower than that of the

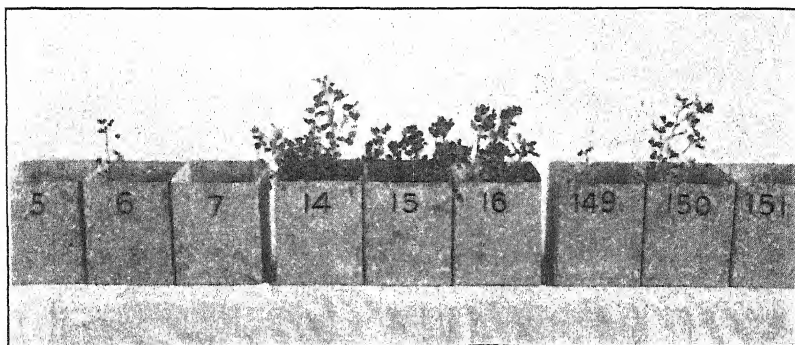


FIGURE 8.—Influence of hardening at different soil-moisture content on the ability of Grimm alfalfa to resist cold. Cans 5, 6, and 7, hardened at 14.3 per cent moisture content and frozen at the same moisture content for 8 hours and 25 minutes; cans 14, 15, and 16, hardened at 14.3 per cent moisture content and frozen at 35 per cent moisture content for 8 hours and 25 minutes. Cans 149, 150, and 151, hardened at 35 per cent moisture content and frozen at 35 per cent moisture content for 13 hours and 15 minutes. At this exposure the minimum soil temperature was the same as in cans 5, 6, and 7.

other groups. In this experiment the freezing exposure was much less severe than in previous experiments, because there was no hardening at low temperatures, consequently this was the only experiment in which freezing was so slight that tops of plants remained uninjured. It is also true that the soil in the low-moisture group was somewhat drier in this experiment than in previous ones.

If the data from all four experiments are summarized certain results are outstanding. Most pronounced among them is the fact that plants growing in soil of low moisture content do not have nearly so good a chance of surviving a short period of cold as do those growing in soil having a higher moisture content. This seems to be due directly to the lower temperature in the low-moisture soil. Any hardening that may occur because of low soil moisture is entirely overshadowed by the difference in soil temperature.

In all four experiments, when the low moisture content was brought up to the high moisture content a slight difference in favor of the low moisture content was indicated in the percentage survival. This may indicate a slight degree of hardening at the low moisture content.

The evidence indicates that a low moisture content of the soil, even to the incipient wilting stage, does not serve to harden alfalfa to any great degree, not nearly enough to overcome the handicap of low-moisture content of the soil during freezing. These results are in agreement with those obtained by Klages (10).

The results no doubt serve to explain certain field observations which indicate that winter injury may be more severe during dry seasons. When it is remembered that actually, under normal conditions, the coldest weather is usually of short duration, it is quite possible that field results approach those obtained in the artificial freezing room.

#### INFLUENCE OF SEVERE WILTING

An experiment was made to determine the influence of severe wilting on the hardening process. In this experiment four flats of each

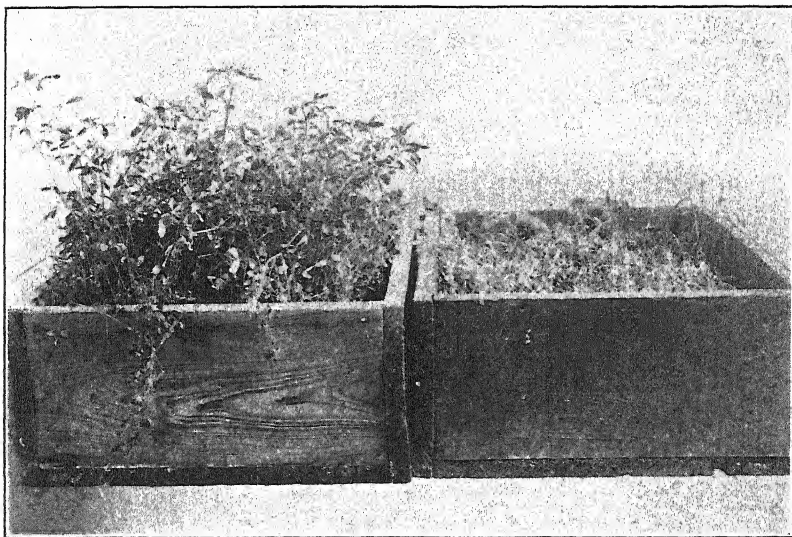


FIGURE 9.—Influence of severe wilting on the ability of alfalfa to resist cold. Kansas common alfalfa was sown in these flats January 30, 1931. Fourteen days before freezing, the flat on the left was allowed to dry, and the plants remained in a severely wilted condition until freezing. The flat on the right was watered normally. Both flats were frozen 48 hours at an average temperature of  $-6.5^{\circ}\text{C}$ . Photographed three weeks after freezing

of three alfalfa varieties—Ladak, Kansas common, and Italian common—were planted January 30, 1931, and allowed to remain in the warm greenhouse. Fourteen days before freezing, half of the flats were allowed to dry. Although some water was added, the plants in these flats were kept in a severely wilted condition until they were frozen. The other flats were watered normally. The plants were frozen May 18, 1931, for 48 hours at  $-6.5^{\circ}\text{C}$ . at the same moisture content that they had carried during the two weeks prior to freezing. This long freeze allowed the soil with normal moisture content to reach the air temperature and thus to reach the temperature of the soil with low-moisture content.

At the end of the 48-hour freezing period the estimated percentage survival of the wilted plants was as follows: Ladak, 85; Kansas common, 90; Italian common, 30. Of the plants that had been normally watered, all were completely killed. Figure 9 shows the difference

three weeks after freezing between two flats of Kansas common treated in the two different ways. The results of this experiment are in accord with those of Rosa (22) in his work with cabbage and of Martin (11) in his work with wheat.

#### RELATIVE IMPORTANCE OF THE INFLUENCE OF LIGHT, TEMPERATURE, AND SOIL MOISTURE ON THE HARDENING PROCESS

An analysis of the results in Table 1 shows that at a temperature of approximately 11° C. a change in length of day from about 10½ hours to 7 hours increased the average percentage survival of Turkestan 56 per cent, that of Grimm 22 per cent, and that of Arizona common 5 per cent. In other words, for each change of one hour in length of day at this temperature and within these limits, the percentage of survival increased by 16, 6.3, and 1.4 for Turkestan, Grimm, and Arizona common, respectively.

Table 6 shows that for a change of 1° C. at any temperature between 12° and 3° the average change in percentage survival is 12 for Turkestan, 10 for Grimm, and 6 for Arizona common.

With respect to moisture content of the soil, no such wide differences are apparent when the actual hardening of the plants is considered, except when the plants are severely wilted. Plants hardened for a period of two weeks near the incipient wilting point, as compared to check plants grown with normal moisture, showed so little increase in survival that no difference due to change in moisture content of the soil could be computed. On the other hand, there was a great difference in survival of plants frozen for relatively short periods at low or high soil-moisture content, as may be seen from Table 11. Arizona common, however, responded to as great a degree as Turkestan, and it is evident that the higher survival due to increased soil moisture during freezing is simply an effect of soil temperature.

The varieties used in the wilting experiment reported in Table 11 do not permit comparison with the light and temperature results; but it is clear that a slight variation in moisture content of the soil at or below the incipient wilting point of the plants exerts a marked influence on the resulting survival. This strong response is apparently not exerted at any soil-moisture content at which the leaves remain turgid.

In comparing the relative influence of light and temperature on the hardening process, it must be remembered that temperatures vary much more than length of day. However, considering the ranges of day length and of temperature that apparently affect the hardening process to the greatest extent, it is evident that both factors exert a very strong influence and that a change of one hour in length of day is very nearly equal to a change of 1° in temperature. Light coming as it does during the warmer portions of the day, appears to be of vital importance in maintaining the correct balance within the plant for optimum resistance. Moreover, it may be noted that varietal response to both light and temperature is very marked. A variety that responds most to change in length of day responds most to change in temperature, and, furthermore, this response is directly correlated with hardiness under field conditions in Nebraska, for the Turkestan variety, F. C. I. No. 15754, used in these tests is one of the most cold

resistant obtainable in that section (19). These data indicate the importance of hardening in bringing out greatest varietal differences in cold resistance.

### DISCUSSION

The reason for the increased hardness in the short-day plants is not clearly understood, but in this connection the work of Nightingale and others is suggestive. Nightingale (15) found that when plants were transferred from long-day to short-day conditions there was an enormous increase in soluble nitrogen in the short-day plants. Harvey (5) found an increase in the amino acid content of cabbage plants on hardening, and Janssen (8) showed some correlation between soluble nitrogen and winter hardness. It should be remembered that in the present experiments the plants were under the short-day conditions only 14 days—insufficient time, perhaps, to reduce the carbohydrates greatly at the low temperatures prevailing. It is also possible that the lack of response to the short day at the high temperatures was due to the rapid utilization of sugars and starches, which would reduce

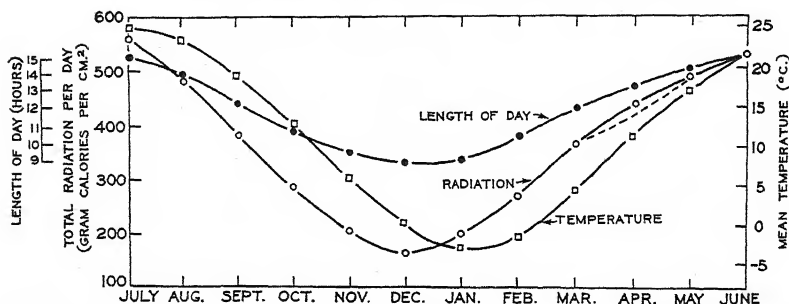


FIGURE 10.—Relation between length of day, average total solar radiation per day, and mean monthly temperature at Lincoln, Nebr., for a period of 12 years

the resistance of the plants to cold. A great decrease in these substances was found by Nightingale in the tomato when plants were transferred from a long day (14 hours) to a short day (7 hours). The low percentage survival of the short-day plants at 25° C. may indicate that photosynthesis was not able to balance the rapid respiration.

The results of these investigations emphasize the remarkable correlation between natural conditions and plant responses. In Figure 10 a striking similarity is observed between the curves for length of day and mean temperature. This figure also shows that temperature lags approximately one month behind length-of-day changes. While the total radiation naturally depends somewhat on the length of day, it also depends on the cloudiness and on the angle of incidence of the sun's rays. Thus, while the length of day varies from 15.1 hours as an average for June to 9.3 hours for December, the solar radiation varies from 566.9 gram calories per square centimeter per day as an average for July to 166.2 gram calories per square centimeter per day for December. If June is considered as 100 per cent in both length of day and solar radiation, December would be 61.6 and 30.4 per cent, respectively.

It may be noticed that solar radiation from the standpoint of time is more closely correlated with length of day than is temperature, and



that, from the standpoint of maximum variation, solar radiation is more closely correlated with temperature than is length of day.

The foregoing experiments show that the optimum response to the short day is at approximately  $10^{\circ}$  to  $12^{\circ}$  C. The days rapidly shorten in October and November, and during this period the average temperature is precisely at this range. (Fig. 10.) Another and perhaps important fact is that while temperatures may vary greatly from day to day and year to year the stimulating effect of the shortening day length on the hardening process is present, and in addition the shortest day lengths precede the lowest temperatures by approximately one month.

An additional similarity between natural environment and the controlled environment found to give optimum hardening is the alternating-temperature effect with light during the warmest portion of the day. It has been noted that under the conditions of this study the alternating temperature of 16 hours in the hardening room and 8 hours in the warm greenhouse during daylight developed greater hardiness in the plants than did continuous low temperatures. Similarly, under natural conditions, the light effect reaches a maximum early in the fall, at relatively high temperatures, and the alternating-temperature effect is greatest later in the fall and in early winter, at lower temperatures.

These studies show very clearly, at least with respect to the three varieties Turkestan, Grimm, and Arizona common, that hardening brings out greater varietal differentiation in resistance to cold.

Since alternating temperatures have been found to exert an important hardening effect, it may be desirable to use controlled alternating temperatures for hardening. Shortened day length for hardening may also be used to advantage under certain conditions. Both temperatures and light are of primary importance in producing optimum conditions for the hardening of alfalfas.

#### SUMMARY

This paper presents a study of the influence of light, temperature and soil moisture on the ability of different alfalfas to survive cold.

Alfalfa plants of the Turkestan, Grimm, and Arizona common varieties, grown for 30 days in the warm greenhouse, were used in the investigation, and a 14-day period was taken as the standard time in the hardening room.

Length of day was found to have a very important influence on the hardening process. The hardier varieties responded markedly to a short day length, under which they hardened off much more than under a normal day length. This response, however, was confined to certain temperature ranges. Practically no response to a 7-hour day at  $20^{\circ}$  or at  $0^{\circ}$  C. was observed, but at a temperature of  $10^{\circ}$  to  $12^{\circ}$  the response was very marked. Of the three varieties studied, the hardiest responded most to the short day; the unhardy responded least. In addition to length of day, intensity of light was found to be an important factor in the hardening process. This was important, however, only when the light reached a minimum beyond which a reduction caused a weakening of the plant. Above this minimum the response to light intensity was not so marked as the response to day length.



The short day at both medium and high temperatures greatly reduced growth. This reduced growth was reflected in a high percentage survival of plants at the medium temperatures, but not at the high temperatures, indicating that reduced growth is not a criterion of hardening.

Plants hardened under white light were better able to withstand cold than those hardened under red or blue light.

The resistance of plants to freezing varied with the temperature at which they were hardened. From 20° to about 0° C. the percentage survival increased as the temperature was lowered. Plants taken directly from a warm room to a hardening room at -5° were killed outright. The greatest response to temperature occurred at about 5°. The varieties again responded in order of hardness, Turkestan showing the greatest increase in hardness for each 1° change in temperature, and Arizona common showing the least.

At the range of optimum activity, light seemed to influence hardening almost as much as temperature, because for each hour of shortening in day length from a 10½-hour day to a 7-hour day there was an increase in survival of 16, 6.3, and 1.4 per cent for Turkestan, Grimm, and Arizona common, respectively, while for each 1° C. of decrease in the hardening temperature the increase in survival was 12, 10, and 6 per cent for the same three varieties. Hardening served to bring out greater varietal differentiation in resistance to cold.

Alternating temperatures during the hardening process markedly increased cold resistance, although this was influenced also by the time of day that plants were subjected to the cold and by the temperature of the hardening room.

Plants subjected to zero temperatures for 16 hours and then placed in a warm greenhouse (approximately 20° C.) during the day for eight hours developed much greater hardness than those kept continuously at 0°. This was true at all temperatures up to approximately 5° for Arizona common and 7° for Turkestan. At these temperatures no increased benefits were obtained by alternating temperatures. At 12° a slight decrease in hardness was found in plants subjected to a high temperature for eight hours during the day.

Two or four hours in a hardening room at 3.8° C. greatly increased the hardness of the plants, the 4-hour plants being about equal in hardness to those kept continuously at 3.8°; but these conditions did not produce as great a degree of hardness as when the plants were kept in the hardening room for 16 hours and the remaining 8 hours in the warm greenhouse in daylight. Two or four hours did, however, give harder plants than 8 hours in the hardening room, when this 8-hour period was taken during the middle of the day and the plants allowed to remain in the warm greenhouse during the night. The least hardy plants were those subjected to the low temperature for 8 hours during the day and for the rest of the time kept in a dark warm room.

Reducing the soil moisture as low as the incipient wilting point did not markedly harden any of the varieties. The rapidity and degree of freezing of the dry soil as compared to the moist soil far overshadowed any hardening effect of the low moisture. The plants in the soil containing the highest percentage of moisture invariably gave the highest percentage of survival when frozen for the same length of time as the low-moisture set. If they were frozen until the soil

temperature in the high-moisture set was the same as in the low-moisture set the survival was slightly in favor of the plants hardened in the low-moisture soil. Plants kept severely wilted for 10 to 14 days and then frozen in the severely wilted condition were very much more resistant to cold than those watered normally.

The close correlation between natural conditions in the fall, such as decreasing day length and alternating temperatures, and the effect on the hardening process of similar factors under controlled conditions is pointed out.

It is suggested that alternating temperatures or decreasing day length, or both, might be used under an artificially controlled environment for hardening plants that are to be frozen by artificially produced low temperatures.

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# LARVAL INSTARS AND FEEDING OF THE BLACK CUTWORM, *AGROTIS YPSILON* ROTT.<sup>1</sup>

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## INTRODUCTION

The studies of the black cutworm, *Agrotis ypsilon* Rott., here described, were undertaken because of outbreaks of the insect which occurred in the "overflow land" of the Black River in the neighborhood of Hoxie, Ark., following inundations that affected thousands of acres of cornland in 1927 and 1928. The experimental work was carried on at the field laboratory of the Bureau of Entomology in Webster Groves, Mo.

## NUMBER OF GENERATIONS

The investigations indicate that there are three generations annually, the adults emerging early in April or May from pupae formed in the fall. The individuals in groups 28727 and 28730 developed from larvae collected in the field in the Hoxie district in 1928. Numerous adults were obtained in October of that year, and some lived until December, but no eggs were produced by them. Those in group 29033-b were from a female captured May 14, 1929. Those in groups 29111 and 29127 were from group 29033-b. Three definite broods were reared from this stock. None of the adults obtained in these experiments survived the winter.

## METHODS

The cages in which the adults were held for oviposition consisted of inverted jelly glasses set in 4-ounce tin boxes, of 2-ounce tin boxes covered by jelly glasses, and of lantern globes with cheesecloth tops and saucer bases.

Eggs were collected daily in the early forenoon, and usually again in the late afternoon, and were kept under frequent observation in closed 2-ounce tin boxes. The newly hatched larvae were caged in 9 by 35 mm culture tubes having tight cotton or cork stoppers. Corn foliage was used for feeding experiments.<sup>3</sup> In the small culture tubes cross sections of corn leaves about one-quarter inch long were used. As the larvae grew and were less likely to be lost, they were placed in 25 by 100 mm shell vials, and when more than half grown were placed in 4-ounce tins. When it seemed apparent that the larvae were

<sup>1</sup> Received for publication July 27, 1932; issued April, 1933.

<sup>2</sup> The writer was assisted by R. C. Lange, assistant scientific aid, and, in the summer of 1929, by James B. Gahan and Dorothy Boyer, in the experimental work described in this paper.

<sup>3</sup> An exception occurred in the night of May 26, when the larvae in group 29033-b were hatching. Each set was given a piece of wheat leaf at 9.10 p. m., which was exchanged for corn foliage by 9.30 a. m. the next day. Micrometer caliper measurements revealed a thickness of 0.09 mm at the edge and 0.11 mm in the blade in both corn and wheat leaf blades, May 27. The quantities eaten were very small and were prorated for each set.

through feeding, sifted soil was added to the tins to permit the larvae to burrow in and form earthen cocoons for pupation.

The partly consumed foliage was removed from the cage once daily and pressed to keep it flat until it could be measured. Rarely, when none had been eaten, the foliage was left another day. In the later instars the supply had to be replenished more than once a day. Each piece of corn leaf blade was cut accurately to a prescribed length and was removed while its outline remained discernible. As the larvae increased in feeding capacity, it was necessary to record the number of pieces given, together with their lengths and widths, as an entire piece might be eaten and thus escape record.

Areas consumed were determined by the use of cross-section paper ruled to hundredths of a square inch. The original contours of pieces of foliage were determined by records and by their remaining edges. By these means the quantity consumed was quickly computed.

Head measurements were made preferably with micrometer calipers adjusted to read 0.01 mm. Measurements made with the aid of the eyepiece of the microscope were less dependable, apparently because of personal error in choosing the plane at which to make the measurement, but this method was used generally until after the second instar. While being measured with the calipers, the larva was held on a moistened camel's-hair brush, when possible, but older larvae were held between the finger and thumb. All measurements were made on the upturn, a procedure which eliminated the error due to the looseness of the caliper screw, as the instrument was set to begin on the upturn at zero.

#### DESCRIPTION OF SEVENTH AND EIGHTH LARVAL INSTARS

Crumb (1, p. 55-56)<sup>4</sup> published descriptions of the first to the sixth instars, apparently having encountered no records of 7-instar or 8-instar larvae. The following descriptions<sup>5</sup> were written from a study of one larva of each of the seventh and eighth instars in life, the specimens being immediately killed and preserved for reference. Ridgway's color key<sup>6</sup> was used for determining the colors recorded.

**SEVENTH INSTAR.**—Head 3.62 mm broad. Extended body 5.48 mm thick, 50 mm long. Cervical shield olivaceous black with narrow median line pale gull gray, and a pale spot at cephalic margin about one-sixth the width of shield from each side. Frons verona brown; major color on epicranium fuscous black on verona-brown background. Ocelli 1, 2, and 5 translucent, appearing whitish; 6 nearly so; 3 and 4 dark. Median line much interrupted, almost wanting on third thoracic and first abdominal segments, vetiver green, as also most of the limited light color of dorsum. Setal spots Ia and IIa blackish slate, each of the II series spots bordered mesad by spots of olive buff. Venter dark grayish olive. Ground color of anal plate dark olive buff, overlaid in part on each side of median line with deep olive. The encircling zone of setal spots III, IV, and V, and setal spot V, pale olive gray, setal spots III and IV olive gray.

**EIGHTH INSTAR.**—Head 3.84 mm broad. Body 5 mm thick, about 45 mm long. Neck full, as if nearing a molt. General color dark olive green with pale olive buff at mesal bases of series of II setal spots, with pale gull gray encircling bases of setae III, IV, and V, with V spot itself gleaming white. Head: Frons and region of eye and antenna verona brown; upper and middle epicranial halves and an area low on side brownish olive, the latter an island on the verona-brown area. Ocelli 1, 2, and 5 translucent, appearing whitish, 3, 4, and 6 nearly the color of the

<sup>4</sup> Reference is made by number (italic) to Literature Cited, p. 530.

<sup>5</sup> The numerals used in these descriptions of the seventh and eighth instars correspond to those used by Crumb in his description of the first six instars and refer to Figures 5 and 13 in the bulletin on tobacco cutworms (7) which figure the larval head shield and setal chart, respectively, for *Feltia* spp.

<sup>6</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

clypeus. Mandibles with four major teeth, the upper somewhat bifid, the lower with a small tooth remote from termination of main tooth. Labrum with two semicircular lobes separated by a narrow rounded notch, paler than frons. The rough epicranium shows the rounded spots far back. Cervical shield olivaceous black with narrow median line hyaline gray, integument of neck pale gull gray. The median line back of cervical shield is broken. The areas representing the greatly reduced subdorsal bands are velvety green, including two broken slender lines on sides. The light area at mesad bases of series II setal spots, the encircling zone of setal spots III, IV, and V, and the V setal spot in its entirety, pale gull gray. Border of median line and dark color of back dark olive gray. I and II setal spots slate black. Setal spots III and IV olive gray. Venter deep slate olive. Anal shield olivaceous black on warm buff, the latter showing in median line and at margins.

The character of skin in the seventh and eighth instars agrees entirely with the description given by Crumb for the sixth instar, except that the "convex, rounded, distinctly isolated, very coarse shiny granules" occasionally range in size up to 0.1 mm each. The adfrontal sutures reach the occipital foramen. Setal tubercle I is about 0.6 mm in longitudinal axis of larva, 0.9 mm wide, and that of II, 0.9 by 1.7 mm.

Setal tubercle I is slightly more than half the diameter, both longitudinal and transverse, of setal tubercle II, each definitely transverse. Tubercle III is slightly larger than I but with less of its surface pigmented. Basal portion of leg claw without tooth. About 20 crochets on each proleg.

## LARVAL DEVELOPMENT AND DETERMINATION OF INSTARS

The development of the larva is indicated in Table 1, in which the head measurements of many specimens are averaged, first for all, then for only those becoming adults, then separately by sexes for individuals which became adults after 6 instars, then separately by sexes for individuals which became adults after 7 instars, and lastly for the few females which became adults after 8 instars. Of those reaching the adult stage, 57.4 per cent passed through 6 instars, 38.9 per cent required 7, and 3.7 per cent required 8 instars.

TABLE 1.—Average head measurements for the entire series and for the individuals of each sex of the black cutworm that matured in 6, 7, and 8 instars, respectively, Webster Groves, Mo.

[illegible]

In the analysis of the average sizes of the head capsule for the several instars, it may be noted that the average of the aggregate in the instar regardless of maturity, and the average of those individuals which became adults regardless of sex or number of instars, check up very closely. The averages for the adults which developed completely in six instars and for those which finished in seven and eight instars are fairly approximate in size, both in the first instar and in the last instar, but they do not check up very closely in the intermediate instars, where the growth of the larvae which underwent seven and eight instars, respectively, was retarded as compared with the growth of the 6-instar larvae. The number of individuals in the 7-instar and 8-instar classes may be too small for consistent record for growth in those classes. In the larger class of the 6-instar individuals the averages are more significant. There is not much difference between the head measurements of the males and females in the first three instars, but after that the heads of the males appear to be generally larger than those of the females.

A further analysis of the head widths is given in Table 2, where the maximum and minimum widths of the head capsules are also shown. For the first and second instars the records cover all the larvae under observation. The measurements given for the remaining instars are of those larvae only which finally became adults.

TABLE 2.—Minimum, maximum, and average head width for the various larval instars of the black cutworm, Webster Groves, Mo.

Instar	Individuals measured	Width of head capsule			Remarks
		Minimum	Maximum	Average	
	Number	Mm	Mm	Mm	
First.....	60	0.26	0.35	0.292	Entire series.
Second.....	120	.36	.52	.442	Do.
Third.....	90	“ .61	“ 1.11	.794	Only those yielding adults.
Fourth.....	92	.77	2.07	1.390	Do.
Fifth.....	96	1.09	2.80	2.140	Do.
Sixth.....	97	1.47	3.94	3.066	Do.
Seventh.....	45	3.14	3.95	3.610	Do.

\* The molts represented by the measurements of 0.61 and 1.11 mm occurred in a period of less than 3 days, during which only 1 examination was made.

The head capsule is the least variable major portion of the larva, and the cast mask is the most durable portion of the exuvium. The head, therefore, is a good guide for determining the instar. While the larva is still active, the ocelli may be observed in the new head behind the ocelli of the mask which is about to be cast. Feeding is suspended, often the major part of a day, before molting. By measuring the head promptly after each molt, and again within two or three days after the last observed molt, the instars may be fairly accurately traced. The observation of the shifting ocelli sometimes aided in detecting a stage of development in the instar after which feeding could not be expected. Suspended feeding before a molt was very often used for fixing the dividing line between the feeding of one instar and that of the next. The actual ecdysis was rarely witnessed.

Measurements were generally made with the microscope eyepiece until after the second instar, because of the difficulty of handling



small larvae when making measurements by calipers. Later instars were measured by caliper.

#### DURATION OF INSTARS

The cages were examined at least once a day, when the food was changed and any exuviae removed and studied, and any details of changes in progress that had taken place since the last examination were noted. Usually the molts occurred between these examinations, and the length of the instar was computed as lasting from an unknown point between two observations to a similar point between two later examinations. For example, an egg hatched between 9 and 9.10 p. m., May 26, and the larva cast its first exuvia between 9.30 a. m., May 28, and 9.30 a. m., May 29; the first-instar period or stadium was, therefore, between 36.33 hours and 60.5 hours. The mean time in this example is 48.41 hours for the duration of the first instar.

Table 3 gives the average range of each instar and of the larval period, by hatching groups, regardless of whether or not the larva reached the adult stage. The first two figures of the group number denote the year. The first two hatching groups from eggs laid September 4 and 5, 1928, hatched between 8.20 and 9 a. m. and 9 a. m. and 4 p. m., respectively, September 10. The third hatching group from eggs of August 27, 1928, hatched between 4 p. m., August 29, and 10.30 a. m., August 30. The explanation for the marked differences in average periods of duration of the first instar is presented under the discussion of effect of temperature. The duration of the instars as recorded may therefore be regarded as fairly representative for the St. Louis district during May, July, August, and September. The first six instars lasted approximately 3, 3, 3, 4, 5, and 7 days, respectively.

TABLE 3.—Record of the duration of instars of the black cutworm for groups hatching at different times, Webster Groves, Mo., 1928-29

Group No.	Time eggs were laid	Total of group	First instar		Second instar		Third instar		Fourth instar	
			Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar
		Number	Number	Hours	Number	Hours	Number	Hours	Number	Hours
28727-----	9.30 a. m., Sept. 4-----	6	6	32 to 55	6	28 to 75	4	35 to 92	3	83 to 140
	8 a. m., Sept. 5-----	10	10	30 to 36	10	32 to 83	10	45 to 99	9	66 to 131
28730-----	4.10 p. m., Aug. 27-----	12	12	255 to 290	11	117 to 176	9	120 to 178	8	118 to 172
		35	35	61 to 96	35	26 to 75	32	41 to 90	31	48 to 93
		4	4	42 to 67	4	19 to 69	3	39 to 88	3	47 to 90
29033-b	2 p. m., May 22, to 3.05 p. m., May 23.	2	4	61 to 86	4	6 to 60	4	23 to 71	4	50 to 96
		2	2	60 to 86	2	*0 to 52	2	23 to 70	2	51 to 95
29111-----	10.30 a. m., July 4, to 9.15 a. m., July 5.	48	48	53 to 89	48	15 to 66	47	33 to 88	42	58 to 112
		37	37	45 to 74	37	32 to 84	35	21 to 83	27	21 to 82
29127-----	10.35 a. m., July 8, to 9.15 a. m., July 9.	11	11	47 to 104	11	16 to 66	9	27 to 73	8	23 to 66
		17	17	27 to 72	17	22 to 74	15	29 to 75	13	25 to 71

\* These larvae were examined May 29, 9.45 a. m., May 30, 11 a. m., and May 31, 1.45 p. m. It is evident that the duration of the second instar was less than 52 hours and that it was more than 0 hour. There are several instances of successive molts on successive days.

TABLE 3.—*Record of the duration of instars of the black cutworm for groups hatching at different times, Webster Groves, Mo., 1928-29—Continued*

Group No.	Fifth instar		Sixth instar		Seventh instar		Eighth instar		Total larval period	
	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of instar	Individuals	Approximate duration of stage
	Number	Hours	Number	Hours	Number	Hours	Number	Hours	Number	Hours
28727.....	3	129 to 172	2	112 to 175	0	-----	0	-----	1	626 to 672
	6	105 to 160	5	125 to 189	2	129 to 193	0	-----	<sup>b</sup> 4	621 to 678
28730.....	6	119 to 181	5	142 to 206	0	-----	0	-----	1	1,008 to 1,051
	29	65 to 110	25	142 to 185	5	147 to 187	0	-----	23	543 to 570
	2	57 to 103	2	110 to 156	1	172 to 212	0	-----	1	1,069 to 1,103
29033-b.....	4	70 to 119	4	106 to 150	2	172 to 213	0	-----	4	536 to 551
	2	70 to 119	2	96 to 147	2	172 to 212	0	-----	2	520 to 543
	40	71 to 111	37	113 to 185	5	178 to 226	0	-----	37	531 to 558
29111.....	25	36 to 84	25	88 to 137	17	151 to 196	0	-----	25	494 to 522
29127.....	8	48 to 98	8	53 to 100	8	280 to 326	3	180 to 218	8	548 to 602
	11	39 to 85	10	49 to 96	9	157 to 202	1	222 to 262	9	400 to 548

<sup>b</sup> These are three 6-instar and one 7-instar individuals.

The mean average duration of larval instars is given in Table 4.

TABLE 4.—*The mean average duration in hours of larval instars of the black cutworm by cage groups for all records in each instar and for those larvae which became adults in the 6-instar, 7-instar, or 8-instar classes, respectively, Webster Groves, Mo.*

Cage group	Series	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Seventh instar	Eighth instar	Total larval period <sup>a</sup>
May.....	Total.....	73	44	62	78	89	140	-----	-----	-----
	6-instar.....	74	42	61	81	99	182	-----	-----	539
	7-instar.....	70	34	56	74	65	89	179	-----	678
July 4.....	Total.....	59	58	57	49	60	113	-----	-----	-----
	6-instar.....	65	61	65	50	81	185	-----	-----	506
	7-instar.....	56	53	43	49	52	84	172	-----	501
July 8.....	Total.....	60	45	51	46	67	74	193	-----	-----
	7-instar.....	58	41	51	41	67	81	197	-----	532
	8-instar.....	54	47	48	48	66	52	78	210	614
August.....	Total.....	272	146	89	145	150	171	-----	-----	-----
	6-instar.....	248	( <sup>b</sup> )	( <sup>b</sup> )	109	95	240	-----	-----	1,030
	Total.....	38	55	68	105	139	154	161	-----	-----
September.....	6-instar.....	47	59	84	176	139	201	-----	-----	647
	7-instar.....	40	23	43	121	146	105	204	-----	681

<sup>a</sup> Not necessarily the total of the mean averages of the individual periods.

<sup>b</sup> Not determined.

A few individuals made records quite at variance with the mean average duration. One larva of the 6-instar class, hatching July 4, required more than 118 hours in the first instar. One larva of the 6-instar class, hatching in May, in the third instar required less than 32 hours.

Less than 49 hours for the sixth instar was required in the case of a larva of the 7-instar class that hatched in May.

There was no eighth instar in the July 4 or August groups. In the September group one larva attained the eighth instar but was killed to represent that instar.

The entire larval period, that is, from the date of hatching to pupation, lasted less than 492 hours in the case of one 6-instar individual, and less than 563 hours in that of one of the 7-instar larvae that hatched in May; less than 457 hours in that of one of the 6-instar larvae and

less than 482 hours in that of one of the 7-instar class that hatched July 4; less than 474 hours in that of one of the 7-instar; and less than 598 hours in that of one of the 8-instar individuals that hatched July 8.

#### EFFECT OF TEMPERATURE

The study of time variations in the development of the immature stages in relation to variations of temperature is on the basis of the published records of the St. Louis station of the United States Weather Bureau. The floor of the thermometer shelter is 737 feet above mean sea level, 10 feet above the main roof of the Railway Exchange Building, and about 264 feet above the street level. The records of temperature taken by the Weather Bureau are used because the laboratory instrument was out of service for several weeks during the period of the experiments.

A cool period from August 30 to September 6, 1928, retarded the first instars of the August group, whereas warm weather following September 10 allowed faster growth, as is shown in Table 3, where the mean durations of the first instar in the August group and September 4 series are, respectively, 272 and 43 hours. The retarding influence of cool weather prevailing when the larvae are in the first instar may, as manifested in this example, be felt throughout the life of the larva. The mean averages for the entire larval periods of the August group and of the September 4 series are, respectively, 1,029 and 649 hours, and for the entire cycle from adult to adult are 1,344 and 1,129 hours. This record of contrasting life-cycle reactions to less than optimum temperature in the first larval instar may account for some outbreak phenomena, and is in keeping with the sensitivity recorded by Crumb (1, p. 54), which appears automatically to preclude the successful hibernation of any forms at Nashville, Tenn., except pupae developing during a short period in the fall.

Two larvae in the September 4 series were followed through the several instars in relation to daily mean temperatures. The mean duration of the first instar and the mean average temperature for the period were, for the first, 40.47 hours, 78° F.; for the second, 63.8 hours, 79.5°. The mean duration of the second instar and the mean average temperature were 46.25 hours, 82.5°, and 90.25 hours, 76.5°, respectively; third instar, 58.05 hours, 77°, and 86.13 hours, 68°, respectively; fourth instar, 170.08 hours, 65°, and 82.75 hours, 61°, respectively; fifth instar, 131.38 hours, 57°; and 131.47 hours, 57°, respectively; sixth instar, identical records, 201.47 hours, 66°. These two records show relatively speedy development with the warmer temperatures in second and third instars, and the reverse in the first and fourth instars; the larvae made identical records in the fifth and sixth instars with the same temperatures, the preceding irregularities having brought them to the beginning of the fifth instar at the same time. This nonadherence to the generally accepted rule of acceleration with warmth and retardation with cooler temperatures is not understood.

#### FEEDING EXPERIMENTS

The feeding records are fairly represented in groups in Table 5. The grouping of feeding records in this table is made according to cage group and hatching date of eggs. The first line of averages concerns all of the individuals in a particular instar regardless of whether they became adults or not. Thus, in the series of 35 hatching in one period

in the May group, only 22 reached the pupal stage in six instars and 3 in seven instars and 1 larva was preserved in its eighth instar. The average for the entire larval period of the group, therefore, could not be obtained because of the irregularities both in number of specimens and of instars and because of the division into the two classes of 6-instar and 7-instar maturing larvae. The average quantities of foliage eaten by all larvae of each of the seven instars, however, are given for the three series of groups.

TABLE 5.—Average quantities of food eaten by larvae of the black cutworm during the various instars, indicated by square inches of leaf surface, Webster Groves, Mo.

Group No.	Date eggs were laid	First instar		Second instar		Third instar	
		Individuals	Foliage eaten <sup>a</sup>	Individuals	Foliage eaten	Individuals	Foliage eaten
		<i>Number</i>	<i>Sq. in.</i>	<i>Number</i>	<i>Sq. in.</i>	<i>Number</i>	<i>Sq. in.</i>
29033-b	May 22-23	35	0.044	35	0.119	33	0.528
		4	.026	4	.079	3	.321
		4	.051	4	.086	4	.300
		2	.026	2	.101	2	.495
29111	July 4-5	54	.042	49	.111	47	.511
29127	July 8-9	5	.046	5	.112	5	.329
		5	.024	5	.128	4	.270
Total or average.		109	.041	104	.112	98	.483
Average quantity of foliage eaten by 65 larvae that eventually became adults.			.037		.114		.540

Group No.	Date eggs were laid	Fourth instar		Fifth instar		Sixth instar		Seventh instar	
		Individuals	Foliage eaten	Individuals	Foliage eaten	Individuals	Foliage eaten	Individuals	Foliage eaten
		<i>Number</i>	<i>Sq. in.</i>	<i>Number</i>	<i>Sq. in.</i>	<i>Number</i>	<i>Sq. in.</i>	<i>Number</i>	<i>Sq. in.</i>
29033-b	May 22-23	32	1.51	29	7.56	26	48.69	3	63.82
		3	1.33	2	4.70	2	31.78	1	61.96
		4	1.36	4	4.24	4	36.36	2	60.36
		2	1.40	2	2.95	2	29.18	1	62.42
29111	July 4-5	43	1.57	40	6.15	38	49.19	6	53.38
29127	July 8-9	3	.68	2	2.61	2	25.29	1	42.88
		2	.95	2	2.57	2	15.65	2	28.70
Total or average.		89	1.49	81	6.27	76	45.85	16	53.57
Average quantity to foliage eaten by 65 larvae that eventually became adults.			1.55		6.01		46.48		<sup>b</sup> 52.53

<sup>a</sup> Average per individual in all cases in the table.

<sup>b</sup> Quantity eaten by 14 larvae.

In Table 6 are recorded the average quantities of foliage eaten in the several instars by all larvae which became adults in six instars, with separate averages for individuals which became males and females, in the May and July 4 groups. Inasmuch as only one individual in each of a number of the groups became an adult, several of the records are those of individuals.

TABLE 6.—Average quantities of foliage consumed by larvae of the black cutworm, May and July 4 groups, that reached maturity after only six instars, and averages by sexes, Webster Groves, Mo.

Record No.	Indi- viduals	Foliage eaten by larvae of the—						
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Total
Larvae of the May group:	Num- ber	Sq. in.	Sq. in.	Sq. in.	Sq. in.	Sq. in.	Sq. in.	Sq. in.
1.....	19	0.044	0.13	0.51	1.71	7.97	55.90	66.26
2.....	1	.032	.07	.47	1.52	7.10	54.66	63.85
3.....	2	.027	.10	.38	1.26	4.62	58.30	64.69
4.....	1	.027	.13	.49	1.43	4.48	48.80	55.36
5.....	27	.035	.12	.50	1.68	6.33	56.61	65.27
Larvae of the July 4 group:								
6.....	1	.070	.20	.66	1.19	3.03	39.61	44.76
Total or average.....	51	.039	.12	.50	1.66	6.79	55.89	65.00
Averaged by sexes:								
Male.....	29	.039	.12	.51	1.69	7.12	52.79	62.62
Female.....	22	.040	.12	.49	1.62	6.35	59.52	68.14

\* The five sets of records for the May group in Table 3 are represented in the five records here given.

In Table 7 are recorded the averages for all larvae yielding adults in seven instars, and the separate averages for individuals yielding males and females, in the May, July 4, and July 8 groups. On an average, the female is decidedly the heavier feeder. The records of feeding in the earlier instars are not constantly heavier for the females, but in the last three instars they are markedly heavier. The duration of the instar, and hence the quantity of food eaten, is dependent largely upon the prevailing temperature.

TABLE 7.—Average quantities of foliage consumed by larvae of the black cutworm, May, July 4, and July 8 groups, that reached maturity after seven instars, and averages by sexes, Webster Groves, Mo.

Record No.	Indi- vidu- als	Foliage consumed by larvae of the—							
		First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Seventh instar	Total
Larvae of the May group: <i>a</i>	<i>Number</i>	<i>Sq. in.</i>	<i>Sq. in.</i>	<i>Sq. in.</i>	<i>Sq. in.</i>	<i>Sq. in.</i>	<i>Sq. in.</i>	<i>Sq. in.</i>	<i>Sq. in.</i>
1-----	2	0.028	0.10	0.53	0.94	4.27	16.38	64.44	86.89
2-----	1	.035	.09	.24	.97	2.30	8.90	61.96	74.49
3-----	2	.074	.06	.22	1.46	3.85	14.42	60.36	80.44
4-----	1	.024	.07	.50	1.38	1.42	9.56	62.42	75.37
5-----	5	.028	.06	.41	1.29	3.38	14.67	52.23	72.07
Larva of the July 4 group:									
6-----	1	.022	.03	.10	.43	2.19	10.97	42.83	56.57
Larvae of the July 8 group:									
7-----	2	.015	.15	.37	.94	2.58	15.64	28.72	48.42
Total or average-----	14	.032	.08	.37	1.14	3.16	14.00	52.53	71.31
Average by sexes:									
Male-----	7	.038	.07	.31	1.14	2.63	11.36	51.93	67.48
Female-----	7	.027	.09	.43	1.14	3.69	16.65	53.12	75.15

\* The 5 sets of records for the May group in Table 3 are represented in the 5 records here given.

The progression in quantity of feeding done in each instar in both the 6-instar and the 7-instar maturing classes is shown in Figures 1 and 2. The averages for the 6-instar class (Table 6) are represented by squares in Figure 1, the outside square representing the total area of foliage consumed. The correct measures of these squares are 0.197, 0.35, 0.71, 1.29, 2.6, 7.34, and 8.06 inches. The averages for the 7-instar class (Table 7) are represented by the squares in Figure 2. The correct measurements of the sides are 0.18, 0.287, 0.61, 1.07, 1.77, 3.74, 7.2, and 8.44 inches, the outside square representing the total quantity eaten.

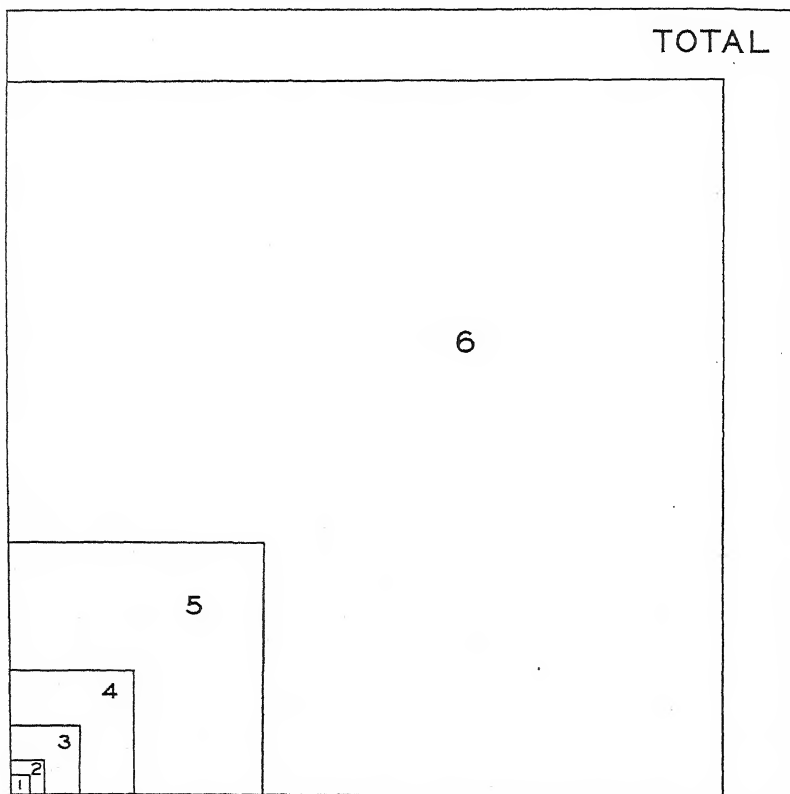


FIGURE 1.—Comparative area of foliage consumed in the successive instars by larvae of the black cutworm that matured in six instars. The numbers in the superimposed squares indicate the instars, and the outside square represents the total area consumed during the entire larval period.

The entire larval period is approximately the same for the 6-instar and 7-instar larvae, but the quantity of food consumed is greater in the 7-instar than in the 6-instar class of the May group. The quantity of food consumed by the 7-instar larvae of the July 8 group is not a satisfactory record, as there were only two specimens in the class. However, the mean temperature for the period of the larval stages of the July 8 group, July 13 to August 3 (81° F.), was 8° higher than for that of the May group, May 23 to June 18 (73°). This would indicate that larvae go through the feeding stage with less aggregate feeding when the weather is warm than when it is cooler.

The progression in quantity of feed consumed and the time spent in each instar are shown graphically in Figure 3. The dotted line represents the May larvae maturing in 6 instars, the solid line the May larvae maturing in 7 instars, and the dash line the July 8 larvae maturing in 7 instars. Only the average duration of the instars and feeding records of the individuals which became adults were used.

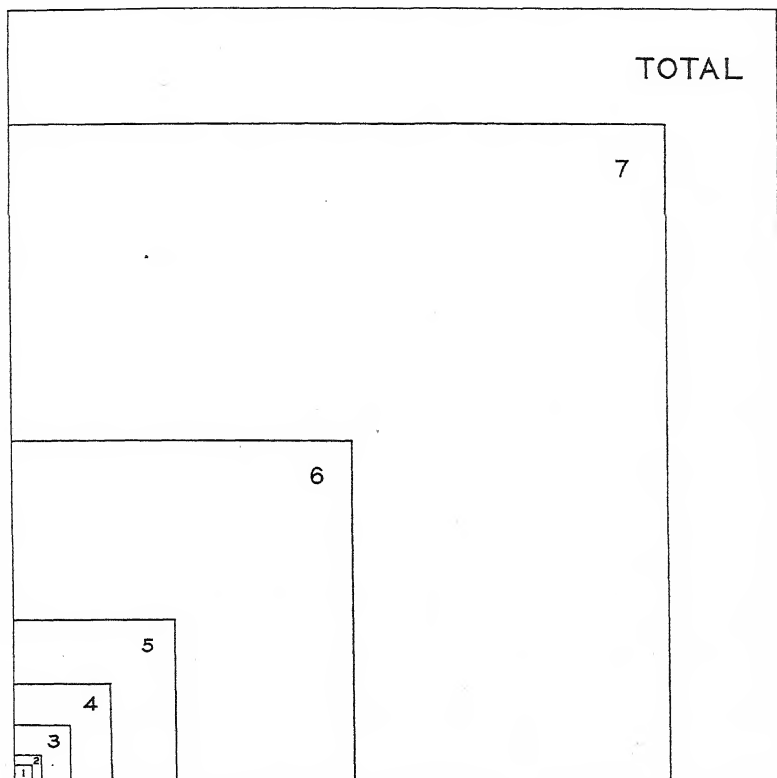


FIGURE 2.—Comparative area of foliage consumed in the successive instars by larvae of the black cutworm that matured in seven instars. The numbers in the superimposed squares indicate the instars, and the outside square represents the total area consumed during the entire larval period

#### DISCUSSION

Garman and Jewett (4) determined that the corn ear worm, *Heliothis obsoleta* Fab., had three complete broods a year at Lexington, Ky., the first brood (July) requiring 12 to 17 days to mature, the second brood (August) 12 to 19 days, and the third brood (September, 1908) 10 to 14 days and (September–October, 1909) 23 to 36 days. The instar periods averaged from 10 records of the first brood were, respectively, 2.63, 2.50, 2.08, 2.42, 2.58, and 3.58 days. The larval development was swiftest in warm weather and slowest in cool weather. The time of development of the last brood was normally lengthened by the cool weather.

Several writers on the instars of lepidopterous larvae of the cutworm family, or of larvae behaving much like the cutworms in their

method of work, including Davis and Satterthwait (2) on the true army worm, *Cirphis unipuncta* Haw., Parker, Strand, and Seamans

(7) on the pale western cutworm, *Porosagrotis orthogonia* Morr., and Luginbill (6) on the fall army worm, *Laphygma frugiperda* S. and A., recorded the duration of the second, third, and fourth instars as shorter than that of the first, and that of the sixth as much longer.

Crumb (1, p. 58) states that in a series of tests on *Agrotis ypsilon* the six instars required, respectively, 2, 2, 3, 4, 4, and 5 days, a shorter duration for the first instar, in relation to that of the second, third, and fourth, than usual, but the usual substantially increased duration for the sixth.

In the present investigation of the black cutworm, effort was made to give all the larvae equally good food. Thus there is no recognized cause for the development of seven or eight instars in the several instances recorded. The record of Decker (3, p. 144) furnishes an entirely satisfactory explanation for the 7 to 11 instars in the 4-lined borer, *Luperina stipata* Morr., because of the continuous feeding of unfavorable parts of the plant to certain individuals. Crumb (1, p. 134, 138) found a seventh instar in the spotted-sided cutworm, *Agrotis badinodis* Grt., and the spotted cutworm, *Agrotis c-nigrum* L., but made no comment.

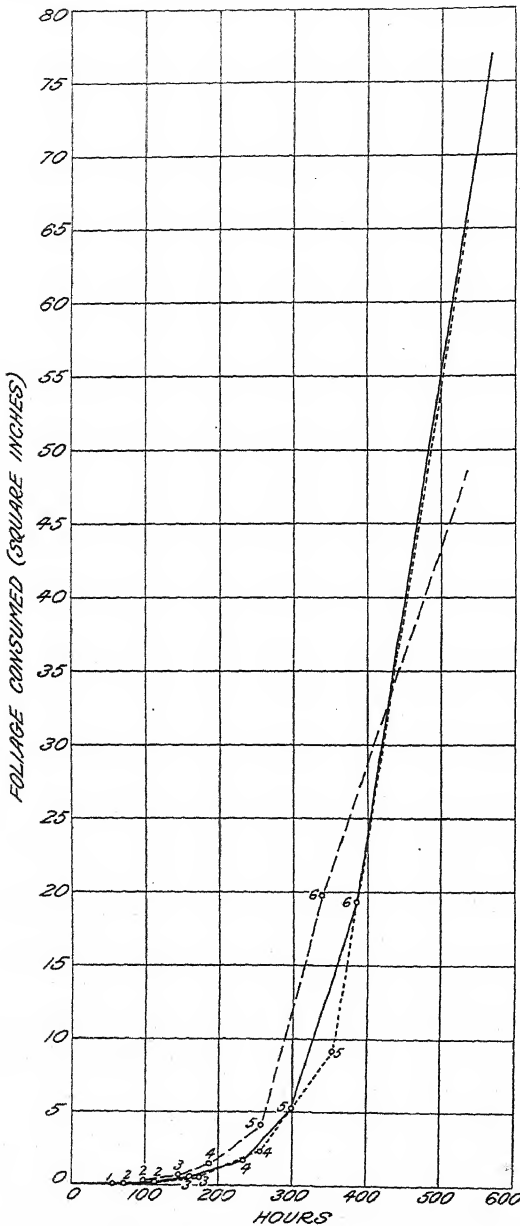


FIGURE 3.—Area of foliage consumed by the black cutworm in various larval instars. The dotted line represents the larvae of the May group maturing after 6 instars, the solid line those of the May group requiring 7 instars, and the broken line larvae of the July 8 group maturing in 7 instars. The figures at points where the lines change direction mark the close of the instars.



It is a common observation that a high temperature speeds feeding and growth and a low temperature checks feeding and growth. The present investigation included temperatures sufficiently low almost to suspend all development for a week at a time. Decker (3, p. 146) used controlled temperatures in certain experiments and reported that—

Larvae reared in an incubator at 27° C. developed almost twice as fast as those reared at 20° C., about two and one-half times as fast as those reared in the screened laboratory, and about four times as fast as those reared at 13° C.

Where there are several generations a year, the duration of the last generation is likely to be lengthened by cooler weather. Garman and Jewett (4, p. 539) found three complete broods and a fractional fourth for the corn ear worm at Lexington, Ky. The three broods required approximately 30 days each. The fourth brood was definitely imperfect on account of frost.

Watson (8, p. 1) indicates that the late fall weather may affect the velvetbean caterpillar, *Anticarsia gemmatilis* Hbn., in the statement that "late in the fall, a few individuals molted seven times."

Parker, Strand, and Seamans (7, p. 299) indicate that the extreme variation in length of life of larvae of the pale western cutworm may be chargeable to food supply rather than low temperature, as the larvae migrate little or not at all, and may remain unharmed through lack of food for several weeks in the feeding season.

Luginbill (6, p. 44) records the fact that the larval period in the fall brood of the fall army worm at Columbia, S. C., averages 29 days, whereas that of the summer brood averages 14 days. This is obviously a slowing down with reduced temperatures.

Holloway, Haley, and Loftin (5, p. 27-28) furnish a tabulated summary showing the numbers of instars of the sugarcane borer, *Diatraea saccharalis* Fab., and numbers of examples in relation to average mean temperatures. Ten instars, the greatest number, was coincident with the lowest average mean temperature occurring in the experiment. Although seven instars occurred at an average mean temperature of 78.9° F., the prevalence of increased numbers of instars at average mean temperatures of 69.8° and lower marks a definite tendency to greater numbers of instars at the lower temperatures. There appeared also to be a slight tendency for instars to increase in number with the highest average mean temperatures. The length of the developmental period of the larvae was great, 242 or more days, at average mean temperatures of 61.4° and lower, and moderate to short, 19 to 76 days, at average mean temperatures of 65.3° and higher.

Crumb (1) shows the lengthening of the larval stage of *Agrotis ypsilon* (p. 57) at cooler temperatures in the fall and of the granulate cutworm, *Feltia annexa* Treit., (p. 79-80). In the variegated cutworm, *Lycophotia margaritosa saucia* Hbn. (p. 112-113), the reaction to cool weather was regular in the tardy larval development in the spring at average mean temperatures of 61° F. and lower, and in the summer, but the larval period lengthened beyond the spring periods at September average mean temperatures not lower than 73.5°.

Watson (8) measured the quantity of food consumed by weighing the foliage before and after feeding, preventing all appreciable evaporation by keeping leaf stems in water, and all under a bell jar.

Davis and Satterthwait (2) investigated the quantities of food consumed by larvae of the true army worm, using 153 individually caged larvae in September and October. The quantity of corn foliage

consumed in the several instars averaged, respectively, 0.03, 0.10, 0.34, 1.20, 5.36, and 34.13 square inches each, an average total of 41.39 square inches for each larva.

Luginbill (6) recorded the quantity of food consumed by larvae of the fall army worm. Two larvae were observed while feeding, corn leaves were used exclusively, and the quantity eaten was measured daily. One larva ate the equivalent of 90.2 cm<sup>2</sup> plus an area of 153 mm<sup>2</sup> skeletonized; the other ate 91.8 cm<sup>2</sup> of foliage and skeletonized an area of 192 mm<sup>2</sup>. The fall army worm thus matures on about one-third the corn foliage required by the black cutworm and requires appreciably less time in the larval stage.

#### SUMMARY

There are three generations of the black cutworm a year at Webster Groves, Mo. Unseasonably cool weather may greatly retard larval development in the first instar and probably serves to increase the mortality rate in later stages of these larvae.

The growth of larvae in general, judged by head measurements, is approximate to one and a half times the size of the preceding head width, if the growth is completed in six instars. Where more instars occur, the increase after the sixth is greatly reduced for the subsequent stages. Increases in size between instars, especially in the first three, may be much greater; rarely the width in the second instar measures twice the width in the first.

The quantities of food eaten in the first three instars are small, then increase abruptly, especially between the fifth and sixth instars, in the individuals maturing in six instars. The measurement of food eaten was possible when corn foliage alone was supplied. In nature, the larvae bite off and then discard entire plants, thus getting an unknown volume of plant tissue and wasting perhaps a thousand times more than they consume. When fed corn foliage exclusively, a larva which undergoes six instars will eat the equivalent of 65 square inches of corn leaf.

The feeding of the larvae which eventually yield females is appreciably heavier than that of larvae which yield males, and is lighter in the July than in the May brood.

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# DEVELOPMENT OF THE NODULAR WORM, *OESOPHAGOSTOMUM LONGICAUDUM*, IN THE PIG<sup>1</sup>

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## PREVIOUS INVESTIGATIONS

Previous to 1925 nodular worms from domestic swine were considered as belonging to only one species, namely, *Oesophagostomum dentatum*, originally described by Rudolphi in 1803. In 1925 Goodey (3)<sup>2</sup> reported the occurrence in swine in New Guinea of a new species of this genus which he named *O. longicaudum*. In the same year Schwartz (6) found specimens of this form in collections of swine parasites from the United States, Tonkin (Indo-China), the Philippine Islands, the Fiji Islands, and China. These findings indicated a rather wide geographical distribution of the new species. In the course of investigations on swine parasites, Schwartz (7) noted that *O. longicaudum* is a common parasite of hogs in the southern part of the United States as far north as Maryland. In that State he found this species to occur rather infrequently, the majority of nodular worms which he collected from pigs raised in Maryland and in the vicinity of Washington, D. C., being *O. dentatum*. In contrast to the rather limited distribution of *O. longicaudum* in the United States, *O. dentatum* was found by Schwartz to be of frequent occurrence in hogs raised in the North as well as in those raised in the South. In addition to the difference in geographical distribution of the two species of *Oesophagostomum* in pigs, Schwartz (7) observed a wide difference in the type of intestinal lesion usually associated with the mature forms of the two species. Small uninfamed nodules, which were usually associated with mature forms of *O. dentatum*, were found in post-mortem examinations of pigs raised in Maryland and the vicinity of Washington, D. C. On the other hand, in post-mortem examinations of pigs raised near Moultrie, Ga., Schwartz observed not only small nodules similar to those found in the intestines of hogs raised in Maryland, but also large raised nodules each surrounded by an inflamed zone. In his discussion of the presence of the large inflamed nodules in southern hogs, Schwartz (7, p. 413) wrote as follows:

The relatively large and conspicuous nodules noted in pigs killed at Moultrie, Georgia, have not been observed in the course of post-mortem examinations of pigs killed in the vicinity of Washington, D. C. Whether this can be accounted for on the basis of the occurrence of species of *Oesophagostomum* in pigs in the South, more particularly *O. longicaudum*, which are absent or rare in the vicinity of Washington, D. C., has not been definitely determined. Fourth-stage *Oesophagostomum* larvae obtained from intestinal nodules of pigs killed in Moultrie and other points in Georgia, have shown a morphology resembling that of the adult forms of *O. longicaudum*, and comparable larvae obtained from intestinal nodules of pigs slaughtered in the vicinity of Washington, D. C., have shown a

<sup>1</sup> Received for publication July 19, 1932; issued April, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 542.

morphology suggestive of that of the adult forms of *O. dentatum*, particularly so far as the shape of the anterior end of the oesophagus and the relative length of the female tail were concerned.

In connection with these findings, the present writer made post-mortem examinations of 367 swine raised in Georgia and the surrounding States and observed that inflamed nodules were present in the intestines of animals infested with *Oesophagostomum longicaudum* alone, as well as in those having mixed infestations. Furthermore, *O. longicaudum* was the most common species of nodular worm found in the animals examined. This species was present in 98 per cent of the animals and *O. dentatum* in 81 per cent. These facts indicated a possible connection between the inflamed nodules commonly seen in southern hogs and the presence of *O. longicaudum* in the intestines of the infested animals, thus validating the suggestion made by Schwartz. In view of the opinion expressed by that writer regarding the economic importance of this pathological condition of the intestines, an attempt was made to obtain definite information on the development of *O. longicaudum* in the intestine of its host and to determine the pathogenesis of the lesion in question. To gain this information a series of infection experiments was undertaken.

#### EXPERIMENTAL PROCEDURE

The infection experiments described in this paper were carried out in Moultrie, Ga., in 1931. Seven pigs 6 to 8 weeks of age which had been raised free from nodular worms were infected experimentally with pure cultures of infective larvae of *Oesophagostomum longicaudum*. The host animals used had been farrowed and raised in a cement-floored pen which was cleaned and then scrubbed with boiling water several times each day throughout the experiments. The efficiency of this method in controlling extraneous infections with intestinal helminths is attested by the fact that no extraneous infections except such as were due to *Strongyloides* were found either in the control animals or in any of the experimental pigs involved in the tests.

Infective larvae of *Oesophagostomum longicaudum* were obtained by culturing ova from gravid females on moist sterile soil at room temperature for a period of five to seven days. The ova were obtained by cutting up the female worms fine with a pair of scissors. At the end of the culture period the infective larvae were recovered from the soil by means of the Baermann apparatus. In view of the fact that Goodey (4) had failed to bring about penetration of the skin by infective larvae of *O. dentatum*, the host animals in these experiments were infected by mouth, 500 to 700 larvae being given to each animal.

The pigs used were killed and examined 48 hours, 10, 17, 35, and 51 days after experimental infection.

#### INFECTION EXPERIMENTS

##### TEST 1

In order to determine the approximate time required by the larvae of *Oesophagostomum longicaudum* to reach the colon and penetrate the intestinal mucosa, the first post-mortem examination was made 48 hours after experimental infection. Numerous petechial hemorrhages were found in the mucosa of the caecum and large intestine.

Each petechial area, when examined under the microscope, appeared to be slightly elevated above the surrounding tissue, thus forming a nodule which contained a depressed area located centrally in the nodule. An early third-stage larva, inclosed in what appeared to be a connective-tissue cyst, was found in each lesion examined. Stained sections of these small nodules showed that the cysts, which were located either in the mucosa or submucosa, were surrounded by areas of intense inflammation with extensive liquefaction of the tissues, particularly between the cysts and the lumen of the intestine. (Fig. 1.) On close examination the cyst walls did not appear to be composed of connective tissue but of a rather tough, somewhat elastic, smooth, homogeneous substance, showing neither striations, nuclei, nor any other indication of cellular organization. In later stages of infection fibroblasts could easily be distinguished clinging to the outside of

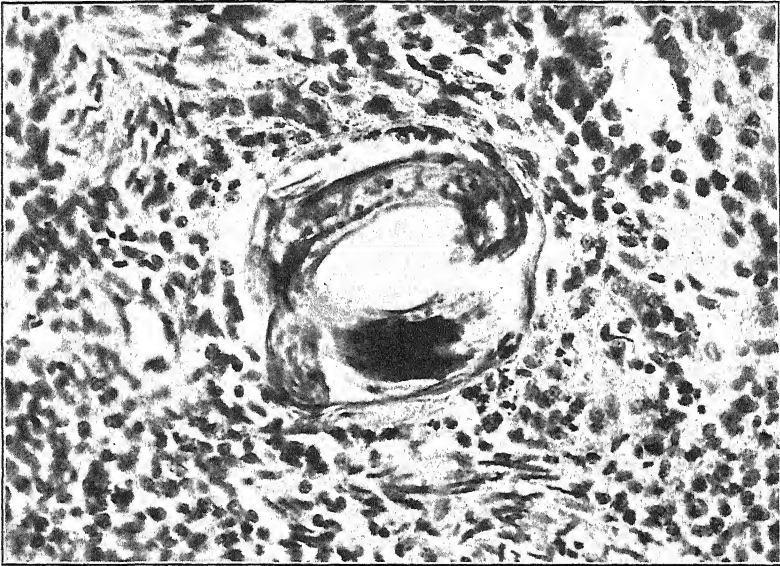


FIGURE 1.—Section through wall of large intestine, showing a cyst containing a larva of *Oesophagostomum longicaudum* 48 hours after experimental infection.  $\times 600$

the cyst walls. As is shown later, even in the case of empty cysts taken from an animal killed 17 days after infection, the cyst walls could easily be distinguished from the surrounding connective-tissue growth by the lack of cellular organization of the former.

The formation of the cysts may perhaps be due to a process similar to that described by Feng (1) in the case of cyst formations in the hedgehog, caused by *Physaloptera clausa*. Around the anterior end of this parasite, Feng observed a liquefaction of the host tissues, resulting in a homogenous zone where the nuclei failed to stain and the borders of the cells were not visible, a condition resembling somewhat the caseous zone caused by the action of tubercle bacilli. It may be that a somewhat similar process takes place in *Oesophagostomum longicaudum* infections. As the larvae penetrate the mucosa of the colon an extensive liquefaction of tissues occurs. The resulting

accumulation of serum, fibrin, and perhaps the remains of partly destroyed cells may undergo a process of coagulative necrosis resulting in a cystlike homogenous zone surrounding the parasite.

In addition to encysted larvae, a small number of third-stage *Oesophagostomum* larvae, which presumably had failed to penetrate the mucosa, were recovered from the lumen of the large intestine.

#### TEST 2

Ten days after infection of a second pig, post-mortem examination of the animal was made to determine the condition of the nodules and the degree of development which the parasites had attained during that period. Nodules raised approximately to a height of 2 mm above the surface of the mucosa and about 5 mm in diameter,

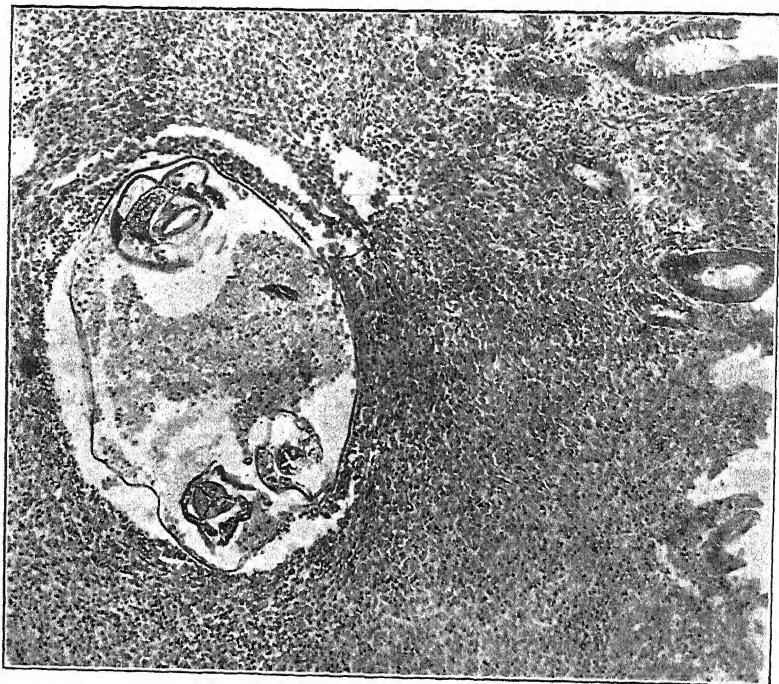


FIGURE 2.—Section through wall of large intestine, showing a cyst containing a larva of *Oesophagostomum longicaudum* 10 days after experimental infection.  $\times 180$

with a central depressed area and a surrounding inflammatory zone, were found in the caecum and large intestine of this animal. In press preparations, late third-stage larvae incased in cysts of a much larger size than those found in the earlier stage of infection could be seen within the nodules. The cysts, inside of which the parasites were freely movable, could be easily dissected out of the nodules. As determined in the course of dissection, the cyst walls were tough and somewhat elastic, with connective-tissue fibers clinging to the outer walls. In sections an extensive destruction of tissue could be seen to have taken place inside the nodules, resulting in what appeared to be a mass of fibrin, coagulated serum, and connective-tissue cells surrounding the cyst. (Fig. 2.)

As in test 1, no evidence could be found in test 2 which pointed to a connective-tissue origin of the cyst, the walls being easily distinguished from the surrounding fibroblasts. As can be seen in Figure 2, the cyst had ruptured in a number of places, perhaps because of pressure by the growing parasite, the gaps being filled, not by connective tissue, but by a substance resembling coagulated serum and fibrin. No appreciable change in thickness of the cyst wall was noted, the increase in size of the parasite perhaps being taken care of by the rupture and subsequent repair of the cyst wall.

No parasites were found in the lumen of the intestine of this animal.

#### TEST 3

On post-mortem examination of a pig 17 days after experimental infection, many fourth-stage larvae and early fifth-stage (mature) parasites were found in the lumen of the caecum and colon. Nodules from which the parasites had escaped were less inflamed, for the most part, and smaller than those that still contained larvae. A photomicrograph of a section through an empty nodule is shown in Figure 3.

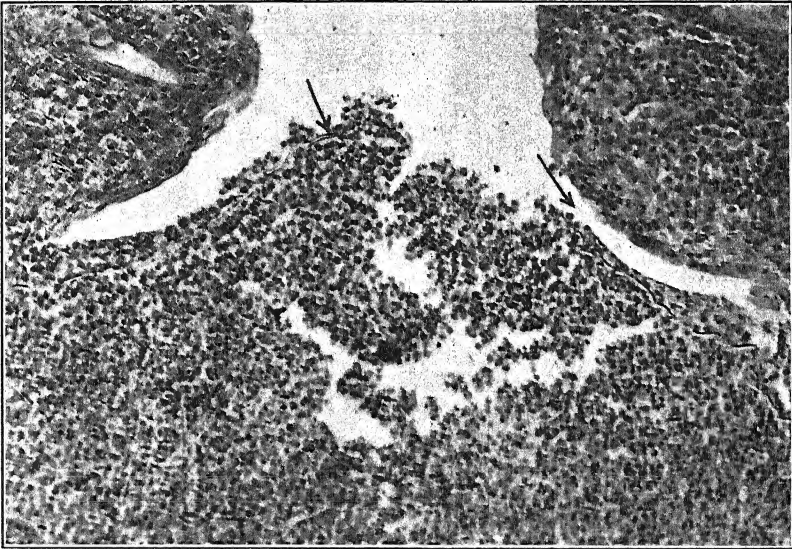


FIGURE 3.—Empty cyst produced by *Oesophagostomum longicaudum* 17 days after experimental infection. Arrows point to rupture in cyst wall.  $\times 180$

The cyst wall is broken, apparently having been ruptured when the parasite emerged from it to enter the lumen of the intestine. After the emergence of the larva the cyst apparently became filled with leucocytes, and the process of absorption of the cyst wall and its replacement by connective tissue, together with the regeneration of glandular tissue, was begun.

#### TEST 4

In a pig examined 35 days after infection, and approximately 18 days after the parasites had begun to enter the lumen of the intestine, the elevated and inflamed condition of the nodules had almost completely disappeared. In the animal examined post-mortem after this



interval, no raised nodules were found, the intestine being dotted with small areas of scar tissue which marked the location of the nodules in the earlier stages of the infection. Figure 4 shows a photomicrograph of a section through a nodule from a pig 35 days after experimental



FIGURE 4.—Transverse section through a nodule in the colon of a pig 35 days after experimental infection with *Oesophagostomum longicaudum* showing the replacement of the cyst by connective tissue. Arrow shows area where cyst was located.  $\times 180$

infection. The cyst wall seems to have been completely absorbed, and only a mass of connective tissue, a few leucocytes, and an occasional giant cell remained to mark the location of the nodule.

#### TEST 5

Three pigs were kept to determine the length of time required for the development of *Oesophagostomum longicaudum* to fertile maturity. By means of the salt flotation technic and the charcoal culture method, the feces of each animal were studied every day, beginning the twenty-fifth day after experimental infection. In two of the animals, eggs were first noted in the feces 50 days after experimental infection. Charcoal cultures of the feces made at this time yielded infective *Oesophagostomum* larvae in seven days when the cultures were kept at room temperature in August. On post-mortem examination of the colon of one of these animals, numerous gravid females were found 51 days after infection. In the third animal, *Oesophagostomum* ova did not appear in the feces until the fifty-third day after experimental infection.



## DESCRIPTION OF LARVAE

## THIRD-STAGE LARVAE

Since the first and second stages of the larvae are preinfective, occurring outside the pig, the present investigation was begun with larvae in the third stage, which is the stage in which they enter the host. No parasitic larvae of this stage were recovered from any of the animals examined post-mortem during these experiments, with the exception of those noted in connection with test 1. These had not progressed, however, beyond the stage of free-living infective larvae. The free-living third-stage (infective) larvae were found to be similar in appearance and size to the corresponding stage of *Oesophagostomum dentatum* as described by Goodey (2). The infective larvae of *Oesophagostomum longicaudum* varied from 645 to 658 $\mu$  in length and were approximately 30 $\mu$  wide. These measurements include those of the sheath.

The infective larva lies free within the sheath, usually with the head pressed closely against the anterior end of this investment. The head resembles a truncated cone and has a terminal depression in which the mouth is located. The diameter of the esophagus is approximately the same throughout its length. The intestine, like that of *Oesophagostomum dentatum*, is composed of 16 cells, 8 on each side. These cells are filled with granular substances, the amount of which seems to decrease with the age of the larvae.

## FOURTH-STAGE LARVAE

On post-mortem examination of a pig 17 days after experimental infection, fourth-stage larvae were found both free in the lumen of the intestine and in the act of emerging from the nodules. Considerable variation was observed not only in size and degree of development, but also in the proportion of length to width of these larvae. The smallest female measured was 2.7 mm long and 306 $\mu$  in maximum width; the largest larva was 5.5 mm long and 288 $\mu$  in maximum width. The males were as a rule smaller than the females, the smallest form being 1.9 mm long and 225 $\mu$  in maximum width; the largest male was 4.2 mm long and 257 $\mu$  in maximum width. Measurements of fourth-stage larvae are given in Table 1.

TABLE 1.—Measurements <sup>a</sup> of fourth-stage larvae of *Oesophagostomum longicaudum*

Item	Measurements of female No.—									
	1	2	3	4	5	6	7	8	9	10
Length of body.....	5.5	4.4	3.9	3.5	3.7	3.6	3.3	3.3	3.0	2.7
Length of esophagus.....	270	293	234	261	266	252	221	270	239	239
Length of buccal capsule.....	36	36	36	36	36	36	36	36	27	36
Distance from excretory pore to anterior end.....	158	162	144	135	149	149	176	144	135	135
Distance from vulva to anus.....	198	270	220	203	212	220	216	203	220	212
Length of tail.....	252	360	270	333	279	279	311	275	270	279
Distance from vulva to anterior end of reproductive system.....	477	887	297	368	90	198	522	293	103	190
Maximum body width.....	288	306	288	329	266	297	221	243	261	288
Maximum width of esophagus.....	68	68	68	54	63	72	68	90	50	90
Maximum width of buccal capsule.....	41	41	41	41	41	41	41	41	32	41
Body width at excretory pore.....	126	162	153	113	126	131	113	117	113	113
Body width at vulva.....	144	176	148	135	144	135	113	126	135	148
Body width at anus.....	68	81	72	77	77	72	86	72	72	72

<sup>a</sup> Measurements of body length are in millimeters; all other measurements are in microns.

TABLE 1.—Measurements of fourth-stage larvae of *Oesophagostomum longicaudum*—Continued

Item	Measurements of male No.—									
	11	12	13	14	15	16	17	18	19	20
Length of body.....	4.2	3.8	3.6	3.4	3.4	2.9	2.6	2.6	2.6	1.9
Length of esophagus.....	284	270	252	252	270	248	293	248	284	270
Length of buccal capsule.....	36	36	32	32	32	36	32	36	36	32
Distance from excretory pore to anterior end.....	203	149	149	158	131	99	117	144	140	122
Length of tail.....	149	153	158	153	167	140	158	135	122	167
Distance from anus to anterior end of reproductive system.....	1,340	1,200	1,370	1,300	1,100	1,179	1,013	1,022	810	900
Maximum body width.....	257	234	261	243	243	239	279	216	252	225
Maximum width of esophagus.....	68	72	68	68	63	54	68	63	54	68
Maximum width of buccal capsule.....	41	41	36	36	36	41	36	41	41	36
Body width at excretory pore.....	117	113	131	122	113	135	122	99	99	126
Body width at anus.....	90	90	99	90	104	99	81	81	59	77

As was observed by Goodey (5) for *Oesophagostomum dentatum*, there is a marked difference in the tails of the two sexes, the female tail being long and tapering gradually, whereas that of the male is short and tapers more abruptly. Both sexes are provided with a provisional buccal capsule which is slightly broader than long, the difference between the width and length being about  $5\mu$  in both sexes. (Table 1.) The walls are thick and heavily cuticularized. (Fig. 5.) Anteriorly each side of the buccal capsule narrows down to a point projecting inward. Posteriorly, after swelling out at first, each narrows to two conical points rather than to one point, as featured by Goodey for *Oesophagostomum dentatum*. These points are directed inward and apparently rest on a heavily cuticularized portion of the anterior end of the esophagus. A

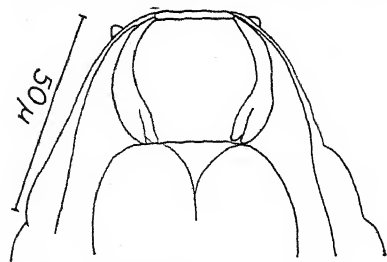


FIGURE 5.—Anterior end of fourth-stage larva of *Oesophagostomum longicaudum* showing shape of provisional buccal capsule.

A single, pointed tooth, apparently arising from the floor of the buccal cavity, projects into the cavity on the dorsal side. Morphologically the esophagus is similar to that of the adult, being more or less swollen at each end. The intestine and rectum are adult in appearance, and the three rectal glands can be seen in both sexes in the vicinity of the junction of the intestine and rectum. A considerable variation in the degree of development of the genitalia of the female specimens was observed. The vagina appears to be formed from a mass of cells, probably ectodermal in origin, lying ventral to the ovejector apparatus, which is similar in shape to that of the adult worm. In the youngest forms studied, the vagina had not opened to the outside. In the larger, more developed forms the vulva had become visible, the opening extending through the cuticle of the worm. From the ovejector apparatus two rows of cells could be seen extending anteriorly to a distance of  $90\mu$  in the youngest specimen and  $887\mu$  in the oldest specimens. Figures 6 to 8 show some of the stages in the development of the female genitalia of this parasite.

The development of the reproductive system of the males of the fourth stage was rather difficult to follow. In all the specimens examined, a long band of cells could be seen lying dorsal to the rectum in much the same position as the spicules of the adult worm; these cells are probably the precursors of the spicules and gubernaculum. (Fig. 9.) The details of the formation of the male genital

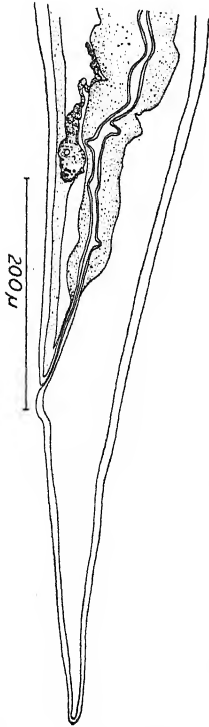


FIGURE 6.—Posterior end of female fourth-stage larva of *Oesophagostomum longicaudum* showing an early stage in the development of the posterior portion of the reproductive system

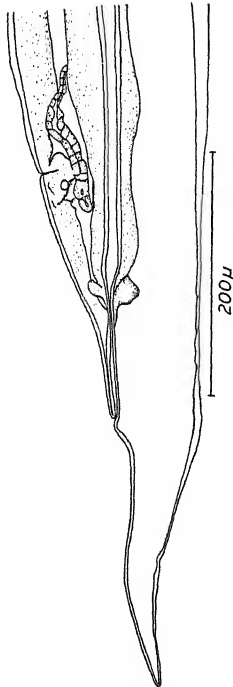


FIGURE 7.—Posterior end of female fourth-stage larva of *Oesophagostomum longicaudum* showing a later stage in the development of the posterior portion of the reproductive system

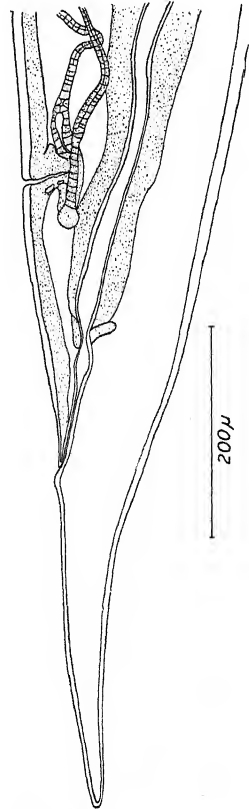


FIGURE 8.—Posterior end of female fourth-stage larva of *Oesophagostomum longicaudum* showing the posterior end of the reproductive system in a late stage of development

apparatus were not fully ascertained. As in the case of *Oesophagostomum dentatum*, a single band of cells could be seen on the ventral side stretching posteriorly from near the genital primordium to a point near the anus, where it turned and extended anteriorly again.

In the specimens studied, the developing bursa could be seen only in outline, its position being dorsolateral to the rectum. The bursal rays could not be clearly distinguished in any of the specimens of the fourth stage which were examined. However, two rows of clear spaces resembling vacuoles could be seen dorsolateral to the rectum;

the bursal rays were apparently formed from the tissue lying between these clear spaces. Figure 9, which is a camera lucida drawing of a developing bursa, gives as much detail as it was possible to make out in an examination of an entire specimen.

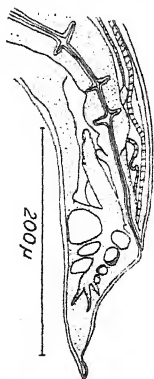


FIGURE 9.—Posterior end of early fourth-stage male larva of *Oesophagostomum longicaudum*

#### FIFTH-STAGE (ADULT) WORMS

Owing to the fact that only two larvae were found undergoing the fourth, or final, molt, the transition from the fourth to the fifth stages could not be definitely traced. The material of the definitive buccal capsule appears to be laid down in the space between the outer border of the provisional buccal capsule and the anterior end of the esophagus. The circumoral papillae appear to be formed in spaces near the middle of the provisional buccal capsule. The beginnings of the internal leaf crown, together with indications of the external leaf crown, can be seen on the anterior border of the developing definitive buccal capsule. Owing to the thickness of material and the large amount of debris through which it was necessary to focus, the exact nature and the relative positions of the adult

characters were difficult to discern. Figure 10, which is a camera lucida drawing of a worm in an early stage of the fourth ecdysis, gives as much detail as it was possible to see. At the base of the buccal capsule, on each side, can be seen a mass of cuticularized material which is probably the developing definitive buccal capsule. On its anterior border the rudiments of the internal leaf crown can be distinguished.

In the late fourth stage of development the female reproductive organs have much the same form as in the adult stage, the principal change at molting apparently being that of size. (Tables 1 and 2.)

As no males were discovered in the process of molting, no description of changes in the male characters can be given.

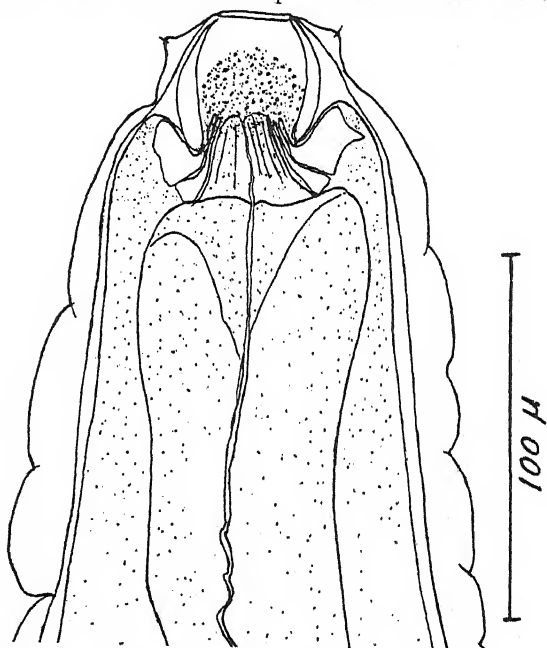


FIGURE 10.—Late fourth-stage larva of *Oesophagostomum longicaudum*, showing provisional buccal capsule together with the developing adult buccal capsule and the internal and external leaf crowns

TABLE 2.—Measurements <sup>a</sup> of fifth-stage worms of *Oesophagostomum longicaudum*

Item	Measurements of female No.—									
	1	2	3	4	5	6	7	8	9	10
Length of body.....	7.8	6.8	5.6	4.5	3.7	3.3	4.1	5.1	2.8	2.5
Length of esophagus.....	391	396	423	432	387	396	404	383	400	378
Length of buccal capsule.....	22	17	17	17	22	17	22	17	17	17
Distance from excretory pore to anterior end.....	168	142	180	180	189	142	142	215	185	180
Distance from vulva to anus.....	357	267	396	258	361	245	387	245	387	387
Length of vagina.....	146	135	135	176	116	135	135	73	135	73
Length of ojector apparatus.....	219	151	206	237	151	151	198	151	241	151
Length of tail.....	487	482	351	360	243	318	353	312	430	306
Maximum body width.....	366	280	288	270	252	280	344	267	318	252
Maximum width of esophagus.....	139	112	108	126	108	90	120	90	112	90
Maximum width of buccal capsule.....	69	65	65	65	65	65	65	69	65	65
Body width at excretory pore.....	185	163	162	144	153	155	172	135	176	153
Body width at vulva.....	224	219	172	144	135	144	172	112	163	198
Body width at anus.....	112	116	90	99	90	56	73	73	86	63
Distance from vulva to anterior end of reproductive system.....	1,022	1,853	1,853	2,788	2,210	1,853	2,210	267	787	787

Item	Measurements of male No.—									
	11	12	13	14	15	16	17	18	19	20
Length of body.....	7.7	6.9	6.5	5.8	5.5	5.4	4.9	4.7	6.3	4.3
Length of esophagus.....	373	378	377	377	361	391	353	370	374	374
Length of buccal capsule.....	22	13	22	17	17	17	22	22	17	22
Distance from excretory pore to anterior end.....	164	181	181	168	181	176	146	133	176	163
Maximum body width.....	308	292	361	280	318	249	206	228	391	224
Maximum width of esophagus.....	103	108	108	103	120	99	99	99	113	103
Maximum width of buccal capsule.....	69	65	73	60	65	65	65	69	65	69
Body width at excretory pore.....	148	163	172	155	172	138	155	133	168	142
Body width at anus.....	127	146	155	181	155	125	133	116	155	99
Distance from anus to anterior end of reproductive system.....	4,318	4,420	3,859	3,366	2,727	3,332	2,890	2,001	4,420	1,904
Length of gubernaculum.....	107	99	108	90	95	86	103	108	90	86
Length of spicules.....	787	851	817	817	817	817	830	839	839	774

<sup>a</sup> Measurements of body length are in millimeters; all other measurements are in microns.

## SUMMARY

Experiments designed to trace the development of the swine nodular worm, *Oesophagostomum longicaudum*, in its definitive host, were carried out by feeding pure cultures of infective larvae of this parasite to pigs raised free from nodular worms.

Forty-eight hours after experimental infection, encysted third-stage larvae were found in the colon of the infected animal; the cysts were surrounded by areas of intense inflammation with liquefaction of tissues.

In sections the cysts, the exact nature of which has not been definitely determined, appeared to be composed of a smooth homogeneous substance containing neither striations nor nuclei, and easily distinguishable from the surrounding tissue.

Inflamed nodules were found in the colon of an animal which had been infected 10 days prior to post-mortem examination. Within the nodules the parasites were inclosed in cysts which were much larger than those found in the earlier stages of infection. The cyst walls appeared to be of the same composition as those observed in the earlier stages of infection and were easily distinguishable from the surrounding fibroblasts. No decrease in thickness of the cyst walls

was noted, the increase in diameter of the cysts apparently being the result of rupture by the parasites, followed by subsequent repair.

On post-mortem examination of an animal 17 days after infection, fourth-stage larvae and early fifth-stage worms were recovered from the lumen of the caecum and large intestine. Late fourth-stage larvae were found in the nodules and in the act of emerging from the nodules, a fact which indicated that the larvae enter the lumen of the colon in the late fourth or early fifth stage of development. Nodules from which the parasites had emerged were smaller and less inflamed than those that still contained larvae. In sections, the cysts inside the empty nodules were seen to be broken, apparently having been ruptured by the parasite when it emerged.

After the emergence of the parasites, the nodules which at first were highly inflamed and contained a central necrotic area, appeared to decrease in size and in degree of inflammation. At the end of 35 days the nodules had almost completely disappeared, their location being marked, in section, by masses of connective tissue, leucocytes, and giant cells.

Sexual maturity, as indicated by the appearance of eggs in the feces of experimentally infected animals, was reached by the parasites in 50 to 53 days after infection.

A brief description is given of the larval stages recovered from the intestines of infected animals.

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# SOME SUMMER-WOOD PERCENTAGE RELATIONSHIPS IN THE SOUTHERN PINES<sup>1</sup>

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## INTRODUCTION

Summer wood, or that portion of the annual ring of wood formed in the latter part of the growing season, has a larger proportion of cell-wall substance per unit of volume than does the remainder, or spring-wood portion, of the ring. This imparts density to the timber and influences its uses. Many of the properties of wood can be correlated with its density as expressed by its specific gravity. Newlin and Wilson (12)<sup>3</sup> have presented such mathematical correlations in great detail. Additional correlations might include qualities of value in the production of wood pulp, such as yield, cellulose content, power and chemical consumption, and paper strength. The fact that the proportion of summer wood varies greatly has long been recognized, but the cause and effect and extent of these variations have had little attention from investigators. Yet this information is important for the most effective growth and utilization of timber.

In most of the investigations to date little has been attempted beyond the establishment of a correlation between width of summer wood, specific gravity, and rate of growth. Hartig (6) apparently was the first to show that the specific gravity of wood is influenced by the proportion of summer wood. Janka (7), working with larch, reached the same conclusion, but he claimed further that there was no relationship between rate of growth and specific gravity. Hale and Fensom (5, p. 18) state that "there is a tendency for white spruce with narrow rings to be heavier than wood with wide rings \* \* \*." Klem (9) likewise points out that ring width is not correlated with specific gravity, especially if the wood comes from different regions with dissimilar growing conditions. He maintains, however, that the specific gravity of the wood increases as the percentage of summer wood in the ring becomes greater. Cieslar (3) noted that in spruce the higher specific gravity was found in wood which contained the greater ratio of summer wood to spring wood. Certain other of his conclusions, as presented later in this paper, indicate that he recognized a relation between rate of growth and density of wood. Paul (13, p. 12) went somewhat farther than the others in his conclusion:

Both very wide and very narrow rings in conifers usually contain a large proportion of the spring-wood layer, so that in these species wood representing either extreme of growth may be low in specific gravity. The wood of intermediate growth is usually the heavier.

<sup>1</sup> Received for publication June 6, 1932; issued April, 1933.

<sup>2</sup> The author is indebted to B. H. Paul, of the U. S. Forest Products Laboratory, for the material which made this investigation possible, and to Arthur Koehler, of the same laboratory, for his helpful criticisms of the paper. The first of the curves was fitted by the class in advanced mathematics at Virginia Polytechnic Institute in the spring of 1930. Dean J. E. Williams was especially helpful in discussing and interpreting the curves, as well as in offering suggestions during the writing of this paper.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 516.

That the specific gravity of the wood of any one species is not necessarily dependent upon the percentage of summer wood is well known. The nature of the summer wood must be taken into consideration, as is pointed out by Koehler (10, p. 131), who emphasizes the fact that "the summer wood itself may vary considerably in density." The radial diameter of the cells (tracheids) in conifers and the thickness of the walls are the main factors influencing the density of this portion of the ring. Adams (1) notes that "the width of the annual ring is dependent upon size as well as number of tracheids." As tracheids increase in radial diameter, the thickness of their walls decreases, leading to a greater lowering of the density than would be anticipated from the increase in tracheid size alone. In addition, the density of the cell wall itself may vary to an appreciable degree, depending upon the amount of infiltrated materials. All of these factors militate against an unvarying correlation between specific gravity and the percentage of summer wood in the ring, but this percentage appears to be the simplest method of determining relative differences in the specific gravity of some species of wood. It is deemed a sufficiently reliable index of density to justify the methods used in this investigation.

Many investigators have shown, as stated by Paul (13, p. 1), that—  
the specific gravity \* \* \* may be modified by controlling local factors which affect the growth either of forest stands or of individual forest trees, so that it becomes possible, within natural limits, to regulate the specific gravity of wood according to the particular use in view.

Paul has shown that trees on burned areas and those on sandy sites consistently produced wood of low specific gravity. The density of stocking was likewise a factor in the weight of wood, since open-grown trees produced lighter wood than crowded ones. Other investigators have found similar effects resulting from density of stocking. Cieslar (3) stated that rapidly growing dominant spruces produced wood of lower specific gravity than did codominant trees in the same stand. But the suppressed or crowded trees were not so consistent. In one instance the wood was higher in density and in another was comparable in specific gravity to that in the dominant trees. Bray and Paul (2) reported that the specific gravity of longleaf pine wood from trees of slow growth averaged 8 per cent higher than that of similar portions of rapid growth, and that, in the same stand, the unit weight of wood increased as the rate of growth decreased because of density of stocking.

That the distance aboveground exerts an influence on specific gravity is likewise evident. Bray and Paul (2) found that the wood of top logs of a medium rate of growth had a lower specific gravity than did any of the butt wood, regardless of its rate of growth. Hale and Fensom (5), on the other hand, noted an increasing density of white spruce wood with increased height aboveground, especially in levels above 30 feet. They suggest, however, that this may be specific for spruce in view of the results obtained by Sterns (15). This author noted a steady decrease in specific gravity in Douglas fir from the butt to the top of the tree.

Paul and Marts (14) concluded, as a result of three years' experiments with the effect of irrigation and commercial fertilizers on longleaf pine, that there was a fairly close correlation between current water supply and the formation of summer wood, that without irri-



gation a complete fertilizer increased the rate of diameter growth because of increases in the spring-wood zone, and a nitrate fertilizer produced diameter increases which were more evident in the summer wood than in the spring wood. Thus it seems to be established that the specific gravity of conifer woods is definitely affected by various factors, among them being position in the tree, density of the stand, and local site factors, such as soil and soil moisture. The present investigation was designed to show in more detail how the variation in the percentage of summer wood, which is responsible for the greatest variations in specific gravity, is brought about.

#### SOURCE OF MATERIAL

The longleaf pine (*Pinus palustris* Mill.) material upon which the greater part of this investigation is based was furnished by the United States Forest Products Laboratory. It had previously been used in another investigation by Bray and Paul (2, p. 163), and the following description is taken from their paper:

The stand selected for study is located near Walnut Hill, Escambia County, Fla., in a former clearing which, according to local residents, was used for farm crops until 35 or 40 years ago. The age of the trees, as shown by the number of annual growth rings on the stumps, ranged from 30 to 35 years. The number of trees per acre varied from 300 or less in some places to about 1,300 in others. The material for this study was obtained from different portions of an area not exceeding 10 acres in extent. This area, comprising the summit of a low, rolling hill, was fairly level. The conditions of topography, soil, and other visible growth factors, except the number of trees per acre, were similar throughout the plots from which the pulpwood samples were cut.

Representative plots from which the wood samples were to be cut were staked out in areas containing approximately 350, 600, and 1,200 trees per acre, respectively.

The trees in the plot containing 350 trees per acre ranged in breast-high diameter from 4 to 14 inches with an average of 8 inches. The trees from the plot with 600 trees per acre ranged from 3 to 10 inches in diameter with an average of 6.4 inches. Trees from the densely stocked plot ranged from 2 to 9 inches with an average diameter of 4.4 inches.

The loblolly pine (*Pinus taeda* L.) material was also furnished by the forest products laboratory. One group (No. 1326) came from Windsor, N. C., and another (No. 1324) from Franklin, Va. These were removed during logging and milling studies, and the density of stocking was not recorded. Since the trees in each group were about the same age, differences in diameter would be indicative of the amount of growing space available. The loblolly pine from each locality was accordingly sorted into two groups. The trees of small diameter were considered as representative of close spacing in the stand, and, conversely, those of large diameter were considered as representative of a more open stand.

#### PROCEDURE

The rings on each section were measured to the nearest hundredth millimeter along a radius from the outside toward the center. A measuring device designed and constructed in the Division of Forest Insects of the United States Bureau of Entomology was used, and the measurements were made continuously from the outside toward the center. In this way any error in measurement or computation could be detected. All curves were fitted by the method of least

squares. When two curves might appear to fit the experimental points the one selected was that in which the algebraic sum of the squares of the residuals most nearly approached zero. In no instance were the curves adjusted in any way.

#### VARIATIONS IN THE TWO PORTIONS OF THE ANNUAL RING

The percentage of summerwood in any one growth ring is the resultant of variations in either or both the spring wood and the summer wood. Thus the variations in the proportion of summer wood dependent upon stocking, position in the tree, and other factors can be interpreted only in relation to the corresponding variations in the width of the ring as a whole.

#### RELATION BETWEEN SUMMER-WOOD WIDTH AND RING WIDTH

No references to the relationship between either summer-wood or spring-wood width and total ring width have been found, except the

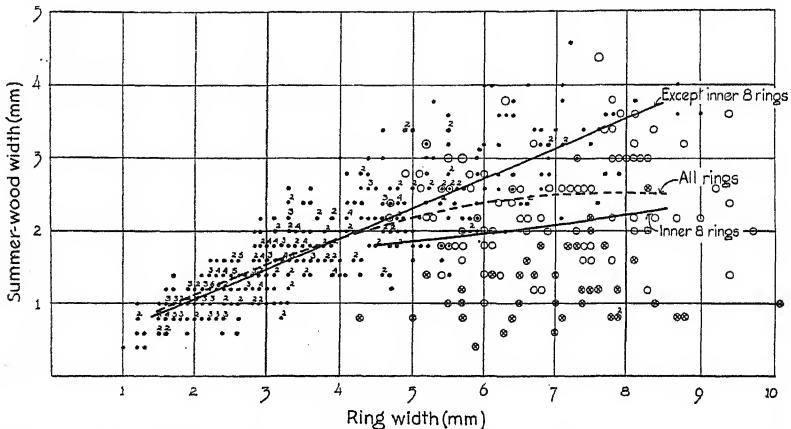


FIGURE 1.—Relationship between summer-wood width and ring width in longleaf pine at breast height in a stand with 350 trees per acre. Points for the eight inner rings represented by circles, for rings outside of the eighth by dots, and for the four innermost rings by crosses within circles. The small numerals indicate identical points

following statement by Forsaith (4, p. 88): "In coniferous species the summer wood is constant in respect to width and such variations as do occur are largely confined to the spring-wood portion of the ring"; and the statement by Paul (13, p. 16) which has already been quoted.

In Figure 1 the summer-wood width has been plotted over ring width for the breast-high sections of longleaf pine with a density of 350 trees per acre. If all of the points are considered, the line around which they group is determined by the equation  $y = -0.05 + 0.652x - 0.041x^2$ . In other words, it is a parabola which is concave downward. In this equation  $y$  represents the width of the summer wood and  $x$  the width of the annual ring.

The general relationship established above for the breast-high sections likewise holds for all of the heights studied in the same trees and for the breast-high sections of longleaf pines from the same tract, but which were grown under different degrees of stocking in the stand. The equations for these other sections are presented in Table 1, and the curves in Figure 2. The relation between summer-

wood width and ring width in loblolly pines from eastern North Carolina and Virginia is expressed by similar curves. (Fig. 3.) Thus it

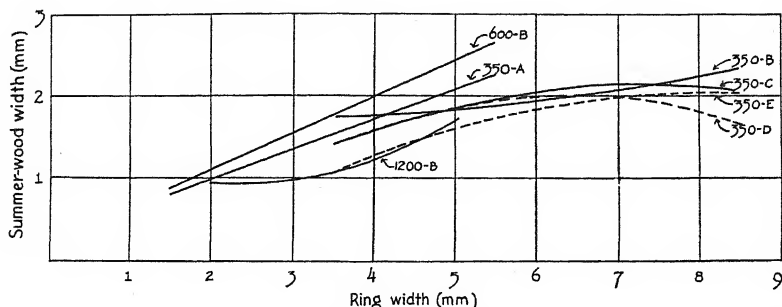


FIGURE 2.—Relationship between summer-wood width and ring width in longleaf pine when all rings on any cross section are included. In the curve designations, the number indicates the number of trees per acre in the stand, and the letter the height above ground, as follows: A, Stump height; B, 4½ feet; C, 8½ feet; D, 12½ feet; E, 16½ feet

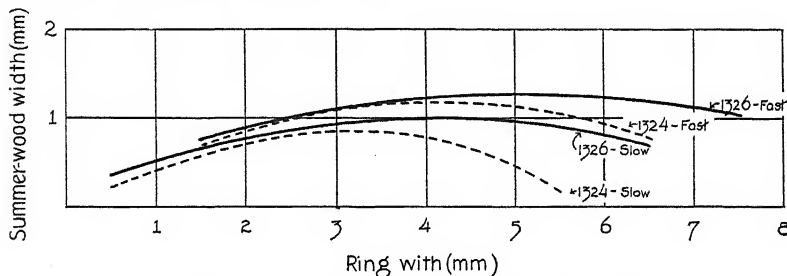


FIGURE 3.—Relationship between summer-wood width and ring width in loblolly pine, when all rings on the cross section 16 feet above ground are included. No. 1326 from Windsor, N. C., and No. 1324 from Franklin, Va. "Fast" indicates light stocking in the stand, and "slow" indicates heavy stocking

is to be expected that a parabola which is concave downward might express the relationship at any height in the southern pines.

TABLE 1.—Equations expressing the relationships between ring width and the spring-wood width, summer-wood width, and percentage of summer wood

Designation <sup>a</sup>	Height above ground	Equations for width of all rings, over ring width		Equations for width of all but 8 inner rings, over ring width	
		Spring wood	Summer wood	Spring wood	Summer wood
	<i>Feet</i>				
350-A	1.5	$y = 0.063 + 0.447x + 0.011x^2$	$y = -0.084 + 0.64x - 0.031x^2$	$y = 0.45x + 0.08$	$y = 0.50x + 0.07$
350-B	4.5	$y = .232 + .265x + .047x^2$	$y = -.05 + .652x - .041x^2$	$y = .55x - .13$	$y = .42x + .23$
350-C	8.5	$y = .634 + .091x + .07x^2$	$y = -.26 + .7x - .049x^2$	$y = .50x + .17$	$y = .42x + .07$
350-D	12.5	$y = .35 + .264x + .056x^2$	$y = -.24 + .69x - .054x^2$	$y = .59x - .07$	$y = .36x + .19$
350-E	16.5	$y = .354 + .315x + .049x^2$	$y = -.06 + .54x - .035x^2$	$y = .61x - .04$	$y = .33x + .21$
1200-B	4.5	$y = .27 + .031x + .14x^2$	$y = .42 + .65x - .086x^2$	$y = .54x - .09$	$y = .35x + .25$
600-B	4.5	$y = .305 + .201x + .061x^2$	$y = .334 + .371x - .003x^2$	$y = .45x + .09$	$y = .46x + .14$
1326-F	16	$y = .481 + .205x + .079x^2$	$y = .22 + .402x - .039x^2$	$y = .69x - .22$	$y = .27x + .285$
1326-S	16	$y = .45 + .176x + .068x^2$	$y = .16 + .408x - .05x^2$	$y = .64x - .1$	$y = .28x + .215$
1324-F	16	$y = .996 - .071x + .115x^2$	$y = -.117 + .642x - .078x^2$	$y = .65x - .08$	$y = .31x + .2$
1324-S	16	$y = -.08 + .627x + .039x^2$	$y = -.03 + .549x - .087x^2$	$y = .71x - .12$	$y = .13x + .38$

<sup>a</sup> Sections 350, 600, and 1,200 are of longleaf pine grown in Escambia County, Fla.; the section numbers indicate the number of trees per acre in each stand. Sections 1326 and 1324 are of loblolly pine, the former grown in Windsor, N. C., the latter in Franklin, Va.; designation "F" signifies fast-growing, lightly stocked stand; "S", slow-growing, heavily stocked stand.

TABLE 1.—Equations expressing the relationships between ring width and the spring-wood width, summer-wood width, and percentage of summer wood—Continued

Designation	Height above ground	Equations for width of inner 8 rings, over ring width		Equations for summer-wood percentage, over ring width
		Spring wood	Summer wood	
	<i>Feet</i>			
350-A.....	1.5	$y = 0.57x + 0.36$	$y = 0.40x + 0.15$	$y = 52.83 - 0.288x - 0.146x^2$
350-B.....	4.5	$y = -.17 + .517x + 0.027x^2$	$y = 1.75 - .045x + 0.013x^2$	$y = 55.26 - .676x - .311x^2$
350-C.....	8.5	$y = 1.39 - .039x + .076x^2$	$y = .085 + .6x - .041x^2$	$y = 51.60 - 1.48x - .167x^2$
350-D.....	12.5	$y = .30 + .36x + .045x^2$	$y = .60 + .79x - .061x^2$	$y = 50.00 - 1.309x - .239x^2$
350-E.....	16.5	$y = .09 + .437x + .036x^2$	$y = -1.08 + .78x - .049x^2$	$y = 42.56 + .212x - .305x^2$
1200-B.....	4.5	$y = 1.86 - 1.19x + .362x^2$	$y = 1.43 - .467x + .103x^2$	$y = 53.29 + 3.8x - 2.06x^2$
600-B.....	4.5	$y = .61x + .07$	$y = .46x + .15$	$y = 51.03 + 1.724x - .761x^2$
1326-F.....	16	$y = 2.883 + 1.513x - .037x^2$	$y = 2.106 - .37x + .034x^2$	$y = 45.12 - .69x - .559x^2$
1326-S.....	16	$y = 1.07x - 2.39$	$y = .01x + .91$	$y = 37.81 + 10.47x - 3.63x^2$
1324-F.....	16	$y = .96x - .6$	$y = .07x + .44$	$y = 41.36 + .034x - .546x^2$
1324-S.....	16	$y = .90x - .3$	$y = .06x + .39$	$y = 39.03 + .73x - 1.214x^2$

While plotting the points, however, it was noticed that in the rings near the center of the trees the relationship between the two variables changed, the points for the inner rings falling below the line established by the rings nearer the periphery of the tree. This departure began somewhere between the fifth and tenth rings from the pith, and varied in different trees. After several trials a dividing line was

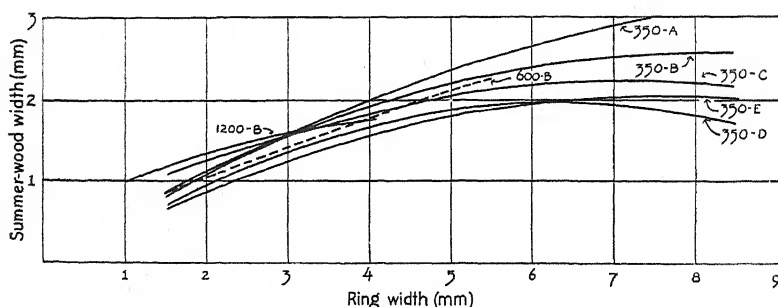


FIGURE 4.—Relationship between summer-wood width and ring width in longleaf pine when only the inner eight rings on any cross section are included. Curve designations as in Figure 2

arbitrarily established between the eighth and ninth rings, and this was used throughout the investigation.

If the curve delimited by the points for the eight inner rings (designated by circles in fig. 1) be determined, it is found to be the parabola  $y = 1.75 - 0.045x + 0.013x^2$ . These data are nearly as well satisfied by the straight line  $y = 0.07x + 1.64$ ; since the algebraic sum of the squares of the residuals is  $-0.0267$  for this curve, and  $-0.0124$  for the parabola. An examination of the trend of points for this group of rings will show that, theoretically at least, it is more nearly expressed by a parabola than by a straight line. It is conceivable, however, that the fewer the wide rings the less reliable are the points at the right-hand end of the curve, and the greater their tendency to be satisfied by the equation for a straight line. This explains why this type of equation adequately satisfies the condition in some of the curves discussed later. Figure 4 shows the trend of the eight inner rings for each of the longleaf pine sections.

Figure 1 shows that the relation between the width of the summer wood and the width of the ring, for the rings toward the outside

of the tree, is represented by a straight line with a marked upward slope. In this case the line is  $y = 0.42x + 0.23$ . This does not agree with the statement made by Forsaith (4) since, if the summer-wood width remained constant, this line should have a slope of zero, i. e., should be parallel to the horizontal axis. Table 1 gives the equations for the same relation in the other longleaf and loblolly pine sections studied. The curves for the longleaf pine sections are presented in Figure 5. In every case studied, the relation is expressed by a similar straight line.

An interesting relation between the slope of these curves is observable. The slope is expressed by the constant accompanying the  $x$  in the equation. If the line were parallel to the  $x$  axis, i. e., horizontal, the constant would be zero; if the line were parallel to the  $y$  axis, the constant would be unity. In the same trees, the slope decreases as the distance aboveground increases, except that the breast-high section shows a slope which approximates that at the  $8\frac{1}{2}$ -foot level. It will be noted later that the breast-high section attained a higher

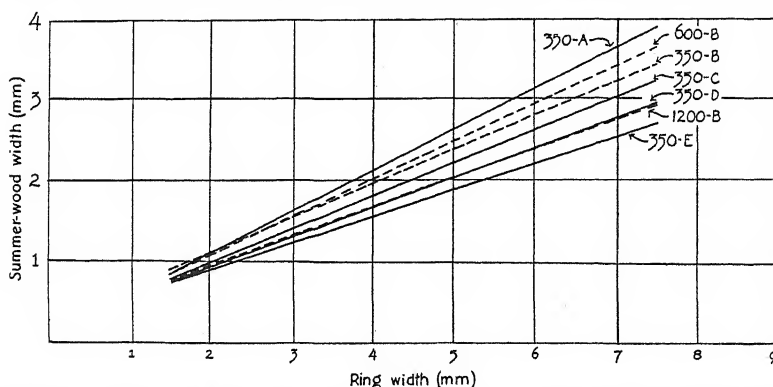


FIGURE 5.—Relationship between summer-wood width and ring width in longleaf pine for all except the inner eight rings on any cross section. Curve designations as in Figure 2

maximum percentage of summer wood than did the  $8\frac{1}{2}$ -foot level. As the density of stocking increases in the stand the slope of the summer-wood-ring width curve increases until the density of 600 trees per acre is reached and then decreases as the number of trees per acre becomes greater.

When it was noted from the foregoing results that the age of the ring entered as one of the variables, 3-plane drawings for each section were made. The data for these were not sufficient to justify the calculation of equations, and only two of the diagrams are presented. Figure 6 shows graphically the relationship between ring width and summer-wood width, as correlated to the number of rings from the center of the tree. The curves representing the relationship between summer-wood width and number of rings from the pith have been smoothed by estimation; those running in the other direction have not been adjusted. These diagrams show the parabolic relationship between ring width and summer-wood width. They also indicate that the curvature in this relationship increases as the rings become wider. At any one age in a given cross section of the trunk it is evident that the summer-wood width increases with ring width. This gives the straight-line relationship already discussed.

## RELATION BETWEEN SPRING-WOOD WIDTH AND RING WIDTH

Since the spring-wood zone is but the difference between the total ring width and the width of the summer wood, the curves representing the relationship between spring-wood width and ring width could be

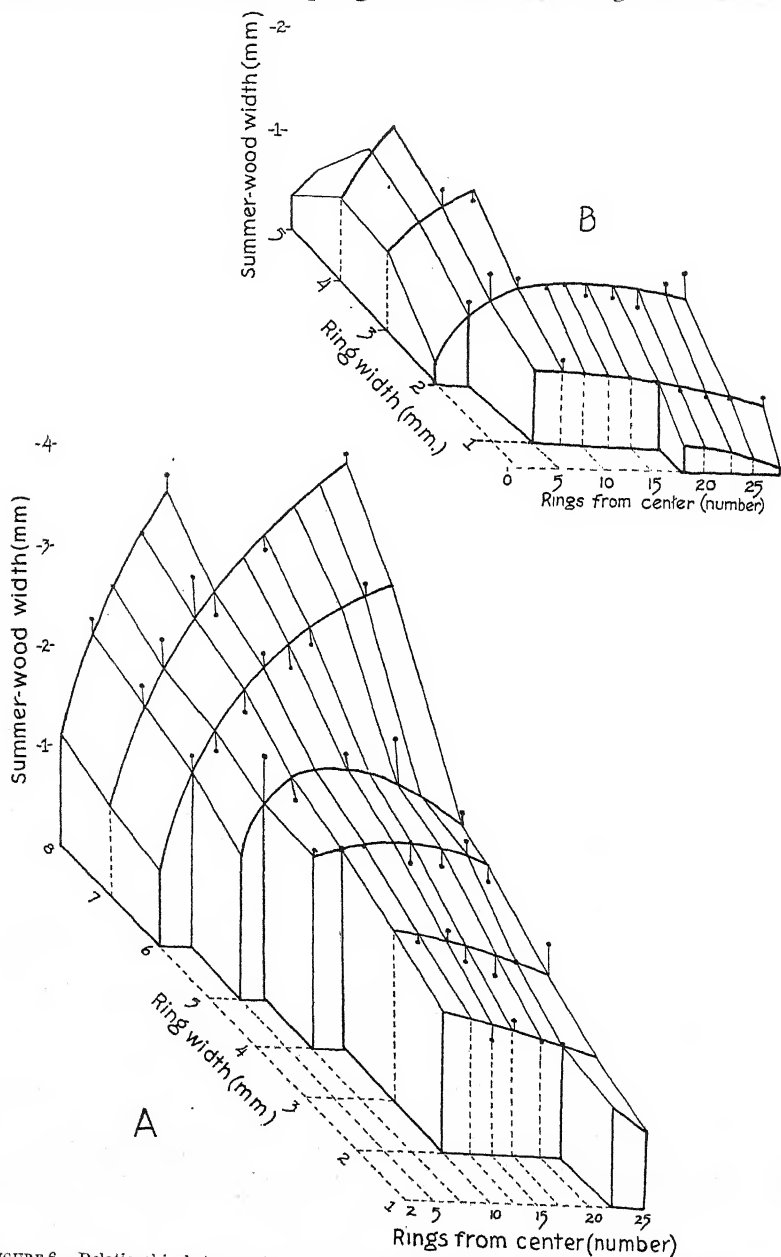


FIGURE 6.—Relationship between ring width and summer-wood width in longleaf pine at different distances from the center of the tree: A, At breast height in a stand with 1,200 trees per acre; B, at breast height in a stand with 350 trees per acre

obtained by subtracting the summer-wood width as read from the curves from the ring width. These data have been computed separately, however, in the same manner as were those for the summer wood, and the equations are presented in Table 1.

As was to be expected, the relation between spring wood and ring width, when all rings on one cross section of the trunk are considered, is expressed by a parabola which is concave upward; when only the eight inner rings are considered, the curve is either a straight line or a parabola; as the rings between the periphery and the eighth ring from the pith become wider, the curve is a straight line with a marked positive slope, i. e., an upward trend; and as the distance aboveground becomes greater, the slopes of the curves for the outer rings increase, except at the 4½-foot level.

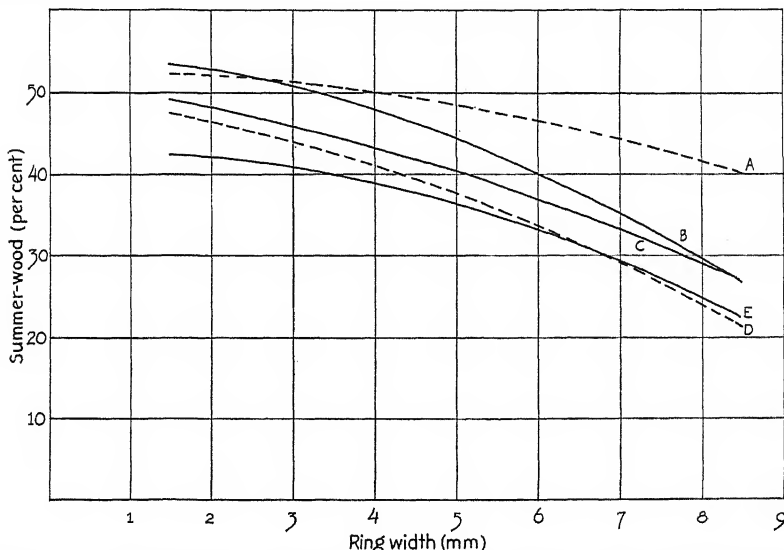


FIGURE 7.—Relationship between percentage of summer wood and ring width at different heights in longleaf pine grown in a stand containing 350 trees per acre. Curve designations as in Figure 2

#### RELATION BETWEEN PERCENTAGE OF SUMMER WOOD AND RING WIDTH

The points obtained by plotting the percentage of summer wood in each annual ring over the width of that ring tend to group around a parabolic curve which is concave downward. This form of curve, as illustrated in Figures 7, 8, and 9 holds throughout all sections of all the pines studied, being modified in the degree of curvature and the maximum percentage attained by both distance above ground and by density of stocking in the stand.

It is possible to vary the degree of curvature of the curves representing the relationship between summer-wood percentage and ring width, by including the wider rings, or, in other words, a larger proportion of the inner rings of any cross section. As the proportion of rings over 10 mm in width becomes greater, the curvature decreases until the data are best satisfied by a straight line with a negative slope, or even by a parabola which is slightly concave upwards. The wide inner rings were so few in number in the trees studied that the data

were deemed unreliable, and in no case have rings over 9 mm in width been used. In several instances, notably in the case of slow-

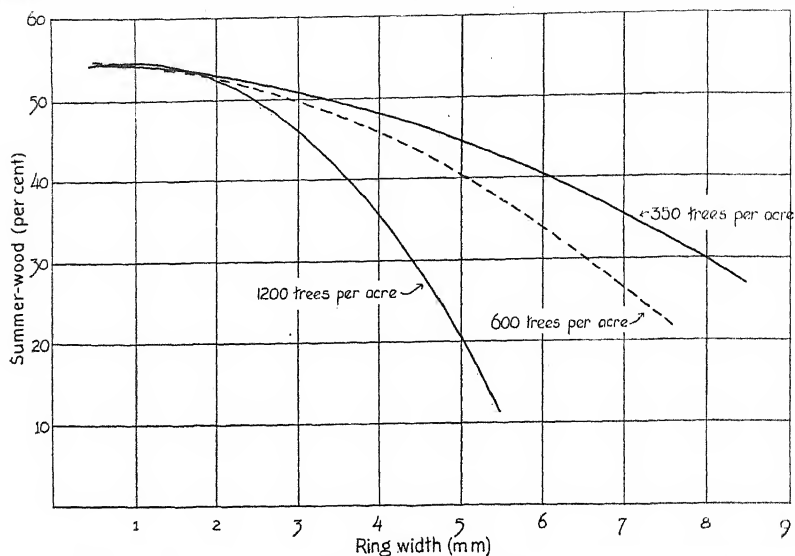


FIGURE 8.—Relationship between percentage of summer wood and ring width at breast height in longleaf pine from stands of different density

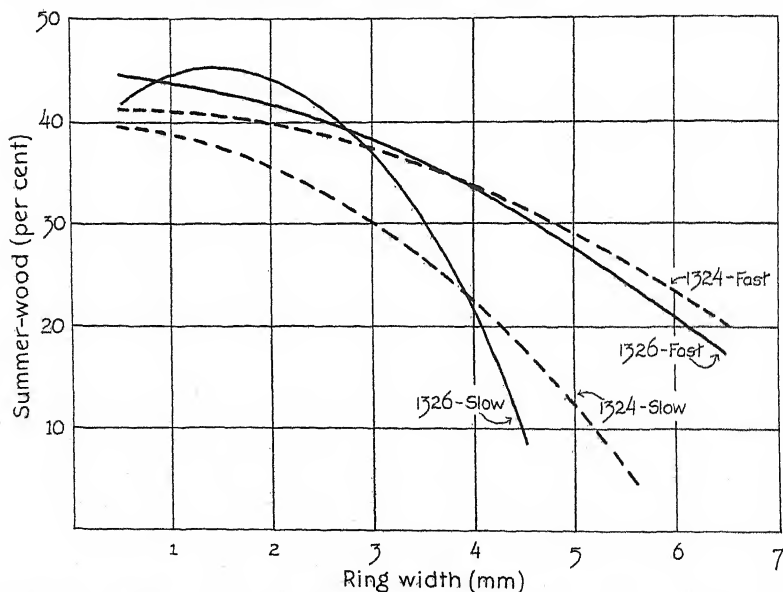


FIGURE 9.—Relationship between percentage of summer wood and ring width at 16 feet above-ground in loblolly pine from stands of light and heavy stocking. "Fast" indicates light stocking and "slow", indicates heavy stocking

growing trees, the maximum reliable ring width was found to be less than 9 mm.

The points have a rather wide dispersion in all the material studied. This can be accounted for only through the variations introduced by



the effect of growth factors on the separate parts of the ring. From the results reported previously by the writer (11) it is conceivable that an annual ring of any given width on any cross section of the tree might evince differences in the percentages of summerwood, depending upon the distribution of the rainfall during the vegetative season. Summer rainfall below the average in total quantity or at infrequent intervals is very likely to result in a smaller percentage of summer wood than if the summer rainfall is normal. Some evidence in support of this was noted while the points for the curves were being plotted. The values for certain years fell rather consistently above or below the average for all years. The writer has also indicated previously that individual trees respond qualitatively to precipitation according to their vigor. Because of irregularities arising from these causes, it is necessary to consider a number of trees, and the greater the number of trees the more accurate the picture presented by the conclusions.

#### EFFECT OF DISTANCE FROM GROUND

The effect of distance from the ground in the same individual was investigated in longleaf pine with a density of 350 trees per acre, since this was the only group for which material at different heights was available. These trees, it will be recalled, were selected from a stand in Escambia County, Fla. Sections were available at stump and breast height and at intervals of 4 feet above the latter point up to 16½ feet. The curves expressing the relationship between percentage of summer wood and ring width for these heights are presented in Figure 7.

#### EFFECT OF DENSITY OF STOCKING

The effect of density of stocking of the stand on the relation of percentage of summer wood to ring width was likewise investigated in the longleaf pine from Escambia County. From curves for the breast-high sections from trees representing the different stand densities, as presented in Figure 8, it is evident (1) that within the limits studied the trees from the denser stands attain a maximum percentage of summer wood nearly 3 per cent greater than do trees from less dense stands; (2) all stands present their maximum density in rings 0.5 mm in width; and (3) the open stands maintain a maximum percentage of summer wood through wider rings than those in which it is found in denser stands. The only other southern pine material available for similar studies was loblolly pine from Franklin, Va., and from Windsor, N. C. Both of these samples were limited in the number of trees represented, as only those of largest and smallest diameter were selected. The former were considered as growing under less crowded conditions than the latter. The curves expressing the relationship between percentage of summer wood and ring width at approximately 16 feet above the ground are presented in Figure 9. Thus in loblolly pine, crowding in the stand exerts the same influence on this relationship as it does in longleaf. The influence appears to be the same whether the trees grew in Florida or in the coastal plain of North Carolina and Virginia. Figure 9 shows also that the relationships which were noted for longleaf pine at breast height hold in their essentials for other heights in the bole of loblolly pine. The densely crowded trees from North Carolina (No. 1326) possessed narrower annual rings than any of the other groups studied. In these trees the

maximum percentage of summer wood was obtained in rings approximating 1.5 mm in width, rather than in rings 0.5 mm or less as in trees of faster growth. The general trend of the curves in Figures 8 and 9 leads to the conclusion that the slower the rate of growth, the wider the rings in which the maximum percentage of summer wood is reached, and the more rapid the decrease in the percentage of summer wood in rings narrower or wider than the point at which the maximum is attained.

A study of Figures 7, 8, and 9 shows that there is no universal relationship between ring width and the percentage of summer wood. The percentage of this thick-walled portion in a ring of any given width depends partly upon the portion of the tree in which this was formed and partly upon the amount of growing space available. That it is also dependent upon site conditions is likewise probable, though the material available during this study did not permit of such an investigation. Keinholz (8) has shown that the average percentage of summer wood in the first 60 rings of lodgepole pine (*Pinus contorta* Loud.) varied with the site. Trees from bogs possessed a maximum of 44.8 per cent summer wood, from virgin growth on good sites 28.5 per cent, and from lava sites a maximum of 22 per cent. Paul (13), as noted earlier in this paper, decided that on better sites the specific gravity of the wood was generally higher.

#### DISCUSSION

One of the outstanding facts all through this investigation was that the general relationships between the two portions of any annual ring and the width of the ring itself hold as well for second-growth loblolly pine as for second-growth longleaf. They also hold whether the loblolly was grown in Virginia or in eastern North Carolina. Preliminary investigations on pitch pine (*Pinus rigida* Mill.) and scrub pine (*P. virginiana* Mill.) from elevations of 2,200 feet in the mountains of southwestern Virginia, indicate identical results. These lend support to the view that the relations here established will be found to hold true generally for second-growth southern pines.

In all of the material studied the inner rings departed noticeably from the relationship established by the rings nearer the periphery of the cross section. As has been previously mentioned, this departure appeared somewhere between the fifth and tenth rings from the pith. It is apparently specific to the individual tree, and does not depend upon distance aboveground or upon amount of growing space. Neither is it caused by the greater average ring width in the inner rings. Figures 3 and 4 show that, where rings of the same width occur in the outer and inner zones of the cross section, the summer wood is almost invariably smaller in proportion in the inner rings. Similarly, as the pith is more closely approached, rings of the same width as found further toward the periphery possess a smaller percentage of the thick-walled summer wood.

The data do not indicate the reason for this departure, but it may be explained eventually on the basis of different metabolic conditions in young stems. The departure from the normal of later life is such as to result in a decreased percentage of summer wood.

It has been shown how the lower percentage of summer wood in the inner rings influences the trend over the cross section as a whole. Whether the straight-line tendency of the relation in the outer rings

between summer wood and ring width and consequently between spring wood and ring width would be maintained if a larger number of wide rings were available in some sections and a greater number of narrow rings in others is problematical. The fact that these curves do not reach a zero summer-wood width when the ring width is zero indicates that the curvature might be modified if a greater number of narrow rings were present. The trend of the points which are so few as to have been deemed unreliable in computing the equations is such as to indicate a probable parabolic tendency. Be that as it may, the reliable data available are best satisfied by straight lines.

From the mathematical standpoint, each of the parabolas expressing the relationship between summer-wood width and ring width may be considered as the resultant of two curves. The first of these is a straight line with a positive slope; the second is a parabola with its vortex at the origin and lying wholly below the  $x$  axis. Since the normal relation between summer-wood width and ring width appears to be expressed by a straight line with a positive slope, the first of these two curves might well be considered as expressing the normal relationship. The second would then be considered as representing the factors which tend to prevent the formation of summer wood. These latter factors exert insufficient control to affect the normal tendency in narrow rings, but become more marked in wide rings and especially in the wide rings near the center of the tree. Further controlled experiments may eventually permit these factors to be determined.

#### SUMMARY

A study of approximately 10,000 rings on second-growth longleaf and loblolly pines from Florida, North Carolina, and Virginia has shown some interesting relationships between the ring width and the percentage of summer wood. The material available has been of such a nature as to permit a study of these relationships under different conditions of stand density, and at different heights in the same individual trees. The following points are deemed to have been established for the second-growth stands studied:

On any cross section the relationship between the percentage of summer wood and the ring width may be expressed by a parabola which is concave downward.

The maximum percentage of summer wood attained at stump height is approximately that attained at breast height in the stand containing 350 trees per acre.

The decrease in the maximum percentage of summer wood attained appears to be more rapid in the uppermost portion of the bole.

The wide rings contain a higher percentage of summer wood at points near the ground.

The maximum percentage of summer wood attained at a given height in the tree appears to be independent of the density of stocking.

The maximum percentage of summer wood at a given height in the bole occurs only in the narrowest rings in crowded stands, but in more open stands this maximum may be maintained in wider rings.

The relationship between summer-wood width and ring width on any cross section is expressed by a parabola which is concave downward when all rings between the pith and the periphery are considered. Conversely, the relationship between spring-wood width

and ring width in the same material is expressed by a parabola which is concave upward.

In the outer rings of a given cross section, i. e., outside the eighth ring, the relationship between summer-wood width and ring width is expressed by a straight line with a positive slope. This is also true for the spring wood.

The relationship between either spring-wood or summer-wood width and ring width in the inner eight rings is variable. It may sometimes be expressed by a straight line and sometimes by a parabola.

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# THE HEMOGLOBIN CONTENT OF THE BLOOD OF HEALTHY AND ANEMIC "SALT-SICK" CATTLE<sup>1</sup>

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## INTRODUCTION

A condition known as "salt sick" affects cattle kept too long on certain range areas in Florida. Loss of appetite, followed by progressive loss of condition even to the point of emaciation, are the most prominent symptoms. Recent investigations (4)<sup>3</sup> have proved this to be a nutritional anemia. The concentration of hemoglobin in the blood of cattle has been studied as an index of the development of or recovery from this condition. Limited data are available concerning the average percentage of hemoglobin in bovine blood, but such variations as might be expected, considering the behavior of the other blood constituents, have never been measured for cattle under uniform conditions of feeding and management. Aston (2), by some iron determinations on the blood of "bush sick" (iron deficient) cattle in New Zealand, has shown anemia to be one of the symptoms of that condition. No direct determinations of hemoglobin were made.

The object of this study was to determine the variations in the hemoglobin content of the blood of cattle observed at 2½-hour, daily, and semiannual intervals, the average values for healthy cattle, and the variations from these values in an anemia due to a naturally occurring iron, or iron and copper, deficiency, such as occurs in salt-sick cattle.

## METHODS OF EXPERIMENTATION

Hemoglobin determinations at the Florida experiment station were made in duplicate by the Newcomer (11) acid-hematin method on blood drawn from the jugular vein. The glass color standard manufactured by Bausch & Lomb was used. Samples were collected in small glass vials containing just sufficient dry sodium citrate to prevent clotting. Analyses were made immediately.

Determinations in field studies were made with a standardized Dare hemoglobinometer (7) on blood obtained by puncturing the marginal ear vein. The same operator made all of the readings reported. All results have been converted to grams of hemoglobin per 100 ml of blood.

It is recognized that the Dare instrument is not so accurate for individual analyses, but in a series of samples the average value

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<sup>2</sup> Grateful acknowledgment is made to tick-eradication officials, Smith-Hughes teachers, and county agents for their assistance in establishing contacts with owners of "salt-sick" cattle; to Dr. C. H. Eckles for permission to obtain data from the Minnesota experimental station herd; to the managers of Florida State institutional herds for cooperation in the study of healthy cattle; and to J. R. Greenman for technical assistance.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 563.

obtained by the Dare method very closely approximates that obtained by the Newcomer method. It is felt that averages of series by either of the two methods can be compared.

### EXPERIMENTAL DATA

#### VARIATIONS IN BLOOD HEMOGLOBIN CONTENT AT 2½-HOUR INTERVALS

Ten Jersey heifers and dry cows were used in this study, the first blood samples being taken at 6 a. m., and successive ones at 2½-hour intervals until 9 p. m. The data are presented in Table 1.

TABLE 1.—*Hemoglobin determinations (Newcomer method) on blood samples taken at 2½ hour intervals from Jersey cattle in 1930*

Date and animal No.	Grams of hemoglobin per 100 cc of blood at indicated time of taking sample								Standard deviation	Coefficient of variation
	6 a. m.	8.30 a. m.	11 a. m.	1.30 p. m.	4 p. m.	6.30 p. m.	9 p. m.	Average		
Sept. 3:										
395.....	6.71	6.22	6.44	6.68	6.04	6.68	6.44	6.46	0.237	3.67
401.....	8.18	9.40	9.05	9.60	9.36	10.24	9.26	9.30	.574	6.17
410.....	8.81	8.90	9.87	9.53	9.67	9.60	9.57	9.42	.373	3.96
Sept. 9:										
295.....	15.90	15.60	15.43	16.23	15.25	16.46	16.12	15.86	.412	2.60
312.....	16.46	15.52	15.34	16.34	15.69	14.73	16.23	15.76	.579	3.67
315.....	14.64	12.98	14.12	14.90	14.38	13.75	15.43	14.31	.740	5.17
334.....	15.52	14.82	14.82	16.46	16.01	15.34	16.12	15.58	.594	3.81
Sept. 11:										
188.....	15.16	13.33	14.38	14.20	14.03	13.89	14.20	14.17	.511	3.61
300.....	12.05	12.91	12.22	12.84	13.61	13.00	12.40	12.72	.496	3.90
305.....	13.47	13.40	13.47	13.33	13.61	13.33	13.68	13.47	.124	.92
Sept. 12:										
295.....	14.90	15.25	15.34	15.25	14.90	15.25	16.12	15.29	.378	2.47
312.....	16.57	14.64	15.08	15.90	14.82	14.99	14.73	15.25	.664	4.35
315.....	13.40	13.75	14.38	14.20	15.16	14.38	12.57	13.98	.769	5.50
334.....	14.20	14.29	15.08	13.89	13.75	14.20	14.20	14.23	.391	2.75
Average.....	13.28	12.93	13.21	13.52	13.30	13.27	13.36	13.27	.162	1.22

Animals No. 395, 401, and 410, were kept in dry lot with water available at all times. The usual ration of silage, hay, and mixed grain was fed. The other animals were on a small Bahia-grass pasture without supplemental feed but with water continuously available. The effect of different methods of feeding should thus be eliminated.

One purpose of this study was to determine whether or not any of the observed variations could be avoided by obtaining the blood samples at some particular time during the day. Assuming that the mean of seven determinations per animal per day represents the value for that day, it would appear from the correlation coefficients between the mean, and the several series of samples, that the series taken at 1.30 p. m. most nearly parallels the mean under the conditions encountered. The next most satisfactory time was 8.30 a. m. The correlation coefficients for the two times were  $+0.9941 \pm 0.0033$  and  $+0.9890 \pm 0.0039$ , respectively.

Apparently there is a tendency for determinations made on early-afternoon samples to give higher results than those obtained on early-forenoon samples. However, the average of the standard deviations for the 14 animals (10 individuals), was  $\pm 0.489$  g and the standard deviation of the means was  $\pm 0.162$  g. This indicates that the variations due to individuality are much greater than those due to time of sampling, and also that individual variations tend to balance each other.

## VARIATIONS IN BLOOD HEMOGLOBIN CONTENT FROM DAY TO DAY

A number of the heifers in the young herd kept in dry lot were bled over periods of 7 to 30 days. Blood samples were taken each morning between 8.30 and 9 o'clock to avoid the possibility of complications due to the time of sampling. The first three animals were under observation during the time of tick eradication<sup>5</sup> and no blood samples were taken on the days when the cattle were dipped. Feeding and management were uniform at all times. The results of this study are presented in Table 2.

No uniformity could be detected in the variations observed. Changes were irregular and no cycles were evident. Several high or low values might, or might not, occur in succession so that day-to-day variations can not be eliminated entirely by taking an average of several successive analyses. The greatest coefficient of variation observed was 9.18 per cent and the average was 5.28 per cent. This variation, as well as that observed during the day, must be considered in the interpretation of the significance of the hemoglobin concentration in bovine blood from time to time.

TABLE 2.—*Variations in hemoglobin content of bovine blood from day to day when animals were bled 7, 10, and 30 days consecutively*

ANIMALS BLED 7 CONSECUTIVE DAYS, BEGINNING JUNE 23, 1930

Animal No.	Hemoglobin per 100 cc blood			Coefficient of variation
	Range	Mean	Standard deviation	
	<i>Grams</i>	<i>Grams</i>	<i>Gram</i>	<i>Per cent</i>
358.....	12.91-14.46	13.65	0.55	4.03
380.....	9.79-10.86	10.18	.37	3.63
382.....	10.62-12.05	11.42	.53	4.64
392.....	9.02-10.20	9.40	.37	3.94
401.....	10.52-11.48	11.06	.35	3.16
405.....	10.11-11.71	11.15	.49	4.39
408.....	9.16- 9.79	9.45	.24	2.54
410.....	7.94- 8.80	8.39	.35	4.17
419.....	8.05- 8.86	8.64	.28	3.24
423.....	7.57- 8.05	7.71	.17	2.20
431.....	8.37-10.52	9.78	.72	7.36
434.....	8.21-10.11	9.13	.59	6.46

ANIMALS BLED 10 CONSECUTIVE DAYS, BEGINNING SEPTEMBER 9, 1931

358.....	12.51-14.03	13.30	0.49	3.68
380.....	9.30-11.19	10.18	.58	5.70
382.....	10.11-11.48	11.01	.36	3.27
392.....	9.23-11.59	10.10	.78	7.72
401.....	9.09-11.10	10.10	.65	6.44
405.....	9.43-12.17	10.70	.68	6.36
408.....	7.66- 8.90	8.14	.36	4.42
410.....	8.12-10.95	9.15	.84	9.18
423.....	7.47- 8.96	7.98	.36	4.51
431.....	7.52- 9.57	8.59	.63	7.33
434.....	8.12-10.90	9.33	.75	8.04
435.....	9.43-12.28	10.35	.78	7.54

ANIMALS BLED 30 CONSECUTIVE DAYS, BEGINNING SEPTEMBER 3, 1930

395.....	6.13-8.86	7.04	0.47	6.68
401.....	7.66-9.68	8.64	.61	7.06
410.....	8.28-9.99	9.05	.44	4.86
Average.....				5.28

<sup>5</sup> All cattle in this area were dipped in an arsenical solution at 14-day intervals, in accordance with State and Federal regulations for the eradication of the cattle-fever tick, *Margaropus annulatus*.

## VARIATIONS IN BLOOD HEMOGLOBIN CONTENT WITHIN A HERD AT SEMIANNUAL INTERVALS

Blood samples were taken from all cattle in the experiment station herd semiannually to test for contagious abortion. Hemoglobin determinations were made at these times on the blood of 21 cows at each of the last four intervals, other cows being eliminated from the tabulation because they were not bled every time. Feeding and management were uniform throughout these years. The average hemoglobin values for the 21 head are as follows:

	Grams of hemoglobin per 100 cc of blood
September, 1930.....	9.77
March, 1931.....	12.34
September, 1931.....	11.00
March, 1932.....	10.12
Average.....	11.31

The highest single determination was 15.25 g, and the lowest 7.92 g. The least variation for an individual for the four bleedings was 0.48 g, and the greatest 6.59 g. Based on the averages for the particular individuals, these variations are equivalent to 5.16 and 64.80 per cent, respectively.

## VARIATIONS IN BLOOD HEMOGLOBIN CONTENT OF NONSALT-SICK CATTLE

To supplement the data in the literature relative to the average concentration of hemoglobin in bovine blood, and to secure data from cattle kept under conditions similar to those encountered in the investigation of a naturally occurring nutritional anemia in Florida, hemoglobin determinations were made on blood samples obtained from two of the State institutional dairy herds in west Florida. Other determinations were made at the Minnesota experiment station, and in some privately owned commercial herds in western Pennsylvania. The numerous determinations of McCay (9) had not then been reported. The data secured, together with a résumé of that in the literature, are presented in Table 3.

TABLE 3.—Average hemoglobin content of bovine blood from areas where nutritional anemia ("salt sick") is unknown

State and county	Investigators	Kind of cattle	Analyses	Hemoglobin per 100 cc of blood			Method used
				Range	Mean	Standard deviation	
			Number	Grams	Grams	Grams	
Florida, Jackson, and Leon counties.		Cows	20	10.99-16.49	14.61	1.45	Dare (7).
		do.	52	10.99-16.49	14.12	1.32	Do.
		do.	60	9.09-14.20	11.06	1.40	Newcomer (11).
Minnesota, Ramsey County.		Heifers.	7	11.40-16.49	14.58	1.68	Dare.
Pennsylvania, Lawrence County.		Cows	39	10.99-16.49	12.68	1.36	Do.
		Heifers	4	12.37-15.11	13.47		Do.
		Calves	3	11.68-15.39	14.06		Do.
		Cows	103		10.94	1.54	Newcomer.
Kansas	Brooks and Hughes (5).	Heifers	59		11.71	1.24	Do.
		Calves	111		10.46	1.65	Do.
		Bulls	24		11.92	1.89	Do.
		Cows			14.10		Tallquist.
New York	Hayden and Fish (8).						
	McCay (9)	Cows and heifers.	1,073		10.90	.86	Cohen and Smith (6).
Pennsylvania	Anderson, Gayley, and Pratt (1).	Cows	5	11.19-15.04	13.19		Van Slyke (12).
		Calves	24	9.70-16.79	12.23		Do.



Values for mature cows using the data of Brooks and Hughes (5), McCay (9), and those presented in this paper, vary from 10.94 to 14.61 g of hemoglobin per 100 cc of blood. Average analyses by the Newcomer (11) and Cohen and Smith (6) methods vary from 10.94 + 1.54 to 11.06 + 1.40 g per 100 cc of blood. The limited data do not permit one to set a similar standard for younger cattle. The calves may or may not have a higher concentration of hemoglobin in their blood than the cows. The heifers, between 1 and 2 years of age, seem to show a higher concentration.

Definite information for young cattle can be secured only by regular periodic determinations of their blood hemoglobin from birth to maturity. The data tabulated make it appear doubtful whether as definite an age trend would be secured as has been given for man. However, these data are sufficient to emphasize the low values sometimes encountered in a naturally occurring iron, or iron and copper, deficiency.

#### EFFECT OF FEEDING IRON AND COPPER SUPPLEMENTS TO SALT-SICK CATTLE

Early in the investigation of salt sick it was noted that the blood was pale in color, and, when centrifuged, usually had less than half of the normal proportion of red corpuscles. The range cattleman's method of curing the condition was to remove his cattle to a different range. Observation showed that healthy ranges were on clay, had a clay subsoil, or were subject to overflow from clay land, while the affected ranges were on white or gray sands, marl soils, or muck lands. There was sufficient forage for the cattle on either type of range, so that quantity of feed available was not a factor.

The similarity of the physical symptoms with those reported for bush sickness (an iron starvation) by Aston (3) in New Zealand, the anemic condition of the cattle themselves, and the apparent scarcity of iron in the soils of the affected ranges, indicated that salt sick might be a form of iron starvation.

TABLE 4.—*Hemoglobin content (Dare method) of freshly drawn blood from cattle on iron, or iron and copper, deficient pastures*

HEALTHY CATTLE				
Age data	Animals	Hemoglobin per 100 cc blood		
		Range	Mean	Standard deviation
	Number	Grams	Grams	Grams
Cattle.....	2	9.62-13.74	11.68	-----
Yearlings.....	28	10.72-16.49	13.34	1.37
Over 2 years.....	4	11.68-14.43	13.57	-----
SALT-SICK CATTLE IN SAME HERDS				
Calves.....	12	3.02- 9.62	5.92	1.62
Yearlings.....	96	3.44-13.05	8.38	2.05
Over 2 years.....	34	4.81-13.19	9.04	2.41
Over 12 years.....	2	-----	4.81	-----
SALT-SICK CATTLE SHORTLY BEFORE DEATH				
Calves.....	9	1.37- 9.62	4.49	2.89
Yearlings.....	7	3.44-12.37	6.62	2.58

Affected cattle were located in 39 herds distributed over the different soil areas where this nutritional anemia was found. Hemoglobin determinations were made on representative affected and healthy animals in these herds before supplemental minerals were provided for cooperative studies. The results of this survey are presented in Table 4.

The values obtained for the cattle which appeared physically fit in these herds, and which the owners considered to be healthy, compare favorably with the average values as given in Table 3. The affected animals had a lower concentration of hemoglobin in their blood than did the healthy animals, although a number of the affected animals would not have been suspected to be iron-deficient, as judged by the concentration of hemoglobin in their blood.

The lowest concentration of hemoglobin observed was 1.37 g per 100 cc in a calf which died, while 3.02 g was the lowest value for an animal that recovered.

The range of the analyses is much greater than is encountered in the normal groups. Apparently there are changes in the blood volume, or other physiological adaptations, that effect the concentration of hemoglobin in the blood stream to such an extent that even after allowing for normal variations it alone can not be regarded as an exact measure of anemic conditions. However, the average condition of a group of animals can be determined very closely by a study of their blood.

Owners of the affected herds were provided with ferric ammonium citrate. This was given as a drench at the rate of 2 or 3 ounces of a 6 per cent solution, according to the size and age of the animal. No other changes were made in feeding or management. Slow and irregular responses to the sole iron supplement suggested that some other element must be deficient also. Cattle at the Florida experiment station (10) made rapid and complete recovery from salt sick when copper sulphate was added to the iron compound in a 1 to 50 molecular ratio. This addition was then made to the supplement used in the field with uniformly favorable results.

Only a single hemoglobin determination was obtained on some of these cattle while as many as six or seven were made on others at approximately monthly intervals. The following tabulation shows the increase in the concentration of hemoglobin in the blood from the time of providing supplement until physical symptoms had almost completely disappeared:

	Grams of hemoglobin per 100 cc of blood
87 head, visibly affected.....	8. 28
87 head, given supplement 1 month.....	10. 61
79 head, near recovery.....	11. 55

#### SUMMARY

Coefficients of variation up to 6.17 per cent were observed for the concentration of hemoglobin in bovine blood when the samples were taken at 2½-hour intervals. The concentration tended to be higher and more regular during the early afternoon and early forenoon. The coefficients of variation, based on 27 series of from 7 to 30 days, average 5.28 per cent. In the same herd under nearly uniform feeding and management, irregular changes in hemoglobin concentration were observed.

The average concentration of hemoglobin in the blood of cows determined by the more accurate methods, ranges from  $10.94 \pm 1.54$  to  $11.06 \pm 1.40$  g per 100 cc.

A study is reported of 39 herds on areas where cattle are affected with a naturally occurring nutritional anemia. Healthy animals in these herds have a normal concentration of hemoglobin in their blood. The lowest value observed in an anemic animal was 1.37 g per 100 cc in a calf shortly prior to death. Values as low as 3.02 g were observed in anemic cattle that recovered from the condition by the use of iron and copper supplement. A total of 79 animals given the above supplement without other change in feed or management regained a normal concentration of hemoglobin in their blood.

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# NORMAL GROWTH OF POTATO LEAVES IN GREENHOUSE AND FIELD<sup>1</sup>

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## INTRODUCTION

This paper records the growth of normal potato leaves in greenhouse and field during the early part of the summer, when optimum conditions for plant growth prevail. The immediate purpose of these studies was to ascertain the period of maximum surface expansion under normal conditions both during the day and during the night. An earlier study of the chloroplasts of the potato (*13*)<sup>2</sup> had shown that the period of maximum growth and division of the chloroplasts coincides with the period of maximum growth of the leaf. A search through the literature revealed few observations regarding the two problems here considered, namely, the size at which potato leaflets show maximum growth in area and the time (day or night) of maximum leaflet expansion.

## REVIEW OF LITERATURE

The older work on leaf growth seems to have been stimulated largely by Sachs' theory of the period of maximum growth (*11*). Stebler (*12*) found that maximum growth took place in *Secale cereale* between noon and 3 p. m. and in *Allium cepa* between noon and 9 p. m., and that daily growth was greatest in the latter plant between the fifth and ninth days at 19° to 21° C. Kreusler's measurements of the area of maize foliage (*8*) indicated a period of accelerated growth at the appearance of tassels and ears. Wildt's measurements of clover leaves (*14*) led him to conclude that sunshine exerted more influence on the amount of actively functioning leaf surface produced than did temperature.

Gregory (*6*), studying the large, irregular, hexagonal to octagonal leaves of the cultivated cucumber, found that only a few of the leaves attained maximum growth at the same time. The rate of growth in a leaf, he believed, "tends to fall off, owing to the action of a detrimental factor."

No data on the growth of the potato leaf were found except those of Bunzel (*1*), who showed that a shoot continues to grow for 67 days, after which the weight of leaves and stalk becomes stationary. Lange (*9*), in a study of the development of chimeras in *Solanum lycopersicum* and *S. nigrum*, found nuclear and cell divisions most numerous between 3 and 5 a. m. In greenhouse plants on the night of March 7, 1925, the mitoses were few in number until 3 a. m.; at 4 a. m. a noticeable increase appeared; and at 5 a. m. the maximum number was present. These results correspond to those obtained by Lutman (*10*) and Karsten (*7*) in their studies of cell reproduction in certain of the algae. Such nuclear and cell divisions do not necessarily mean a marked increase in the bulk of an organ.

<sup>1</sup> Received for publication Dec. 7, 1931; issued April, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 577.

In the mature leaves used by Denny (4) a gain in fresh weight and a loss in dry weight occurred during the night, indicating the absorption of water and the translocation of some of the materials, especially carbohydrates, and possibly nitrogenous compounds.

Davis and Sawyer (3) found sucrose present in the leaves in increasing amounts from sunrise to 2 p. m., but after that hour it decreased. Hexose sugars were also present, but the percentage varied owing to the formation or solution of the starch. From about 2 p. m. until late afternoon hexoses were abundant in the leaves, owing probably to conversion of the sucrose present. In the leafstalks reducing sugars predominated over sucrose. Storage of starch, hemicelluloses, sucrose, and dextrose are also recorded by Clements (2). He found the maximum at 6 to 8 p. m., with sucrose or dextrose present at all hours of the day and night, although not in very large quantities.

### METHODS

The measurements herein recorded were made at the Vermont station on plants of Green Mountain potato (*Solanum tuberosum* L.) growing in pots in the greenhouse and in the open. No special effort was made to control temperature, humidity, or the length of day under which the plants were grown. Controlled experiments, while of scientific value, were entirely apart from the purpose for which these records were made. The temperature in the greenhouse never went below 60° F. at night but rose to above 100° in direct sunshine. The plants, in 5 to 6 inch pots containing rich greenhouse soil, were placed at night in a box electrically heated by resistance coils, which raised the night temperature in the box about 5°. The plants grown in the field were also in pots. The temperature, although the experiments were made in the latter part of July, fell to almost 50° on one of the nights. The plants were about 6 to 7 cm high when leaflet measurement began, and had five to six leaves. All branches except one were removed. The measurements of length and breadth were made with narrow strips of millimeter paper and were accurate to within about one-half millimeter. The very young leaflets open out in a fan-shaped figure, with the leaf lamina decurrent on the petiole, so that the measurements of length are not so accurate for the first two or three days after the leaflets appear as they are later. When the leaflets are 1 cm in length they flatten out and are as easy to measure as a piece of paper of the same size.

Certain selected leaves were measured as soon as they began to make rapid growth, the object in this case being not to trace the growth of the leaf but to determine the maximum increment of growth made during the day and during the night. Measurements made at 7 to 8 a. m. and 7 to 8 p. m. were plotted.

The leaves were reproduced to scale on coordinate paper, the daily or half-daily increments being added and a mature leaflet of the correct form and measurement built up. These areas were then determined with a planimeter. In view of the regular form of most of the potato leaflets this method should give results the error of which would not exceed 5 per cent.

## GROWTH OF A SINGLE LEAF

The results of the measurement of a single typical leaf (sixth from the bottom, with four leaflets) from a greenhouse plant are shown in Figures 1 and 2. Leaflet A grew rapidly during the first eight days of recorded measurement, but growth declined after May 16, the daily

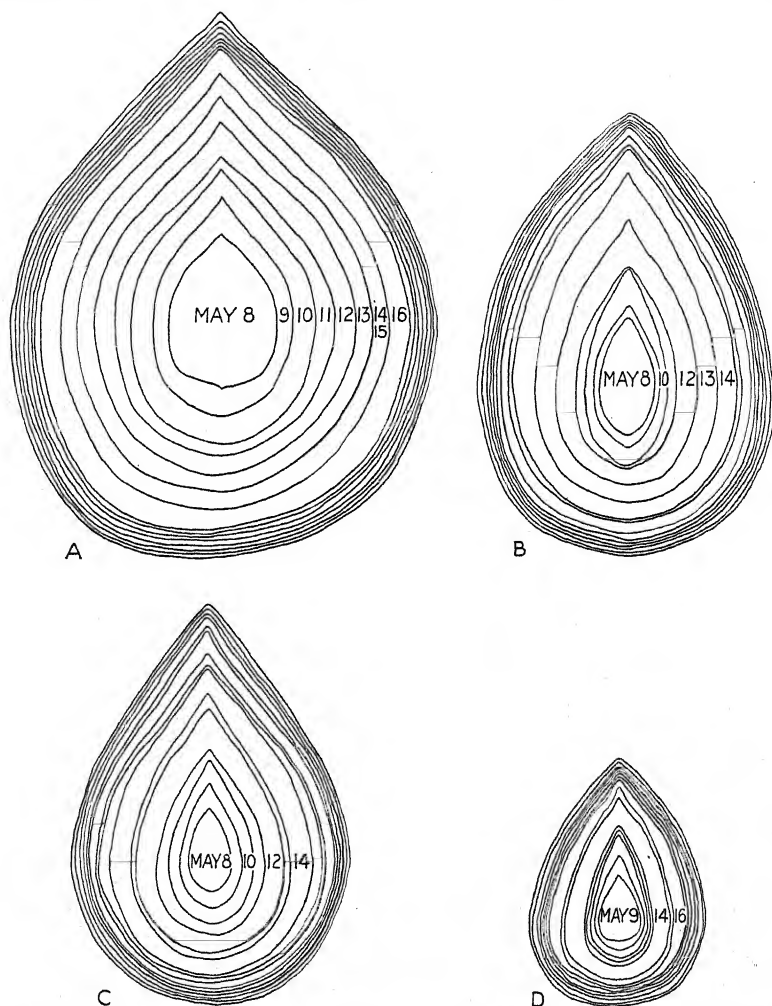


FIGURE 1.—Growth of the four leaflets on a typical leaf (No. 6 on the stem from the bottom), from May 8 to May 25, in the greenhouse

increase dropping abruptly from 23 per cent on May 15 to an average of 3 per cent for the remaining nine days. In leaflet B permanent slackening in growth occurred on May 17, the daily increase dropping from 15 per cent to an average of 3 per cent for the remaining eight days. Likewise, marked decrease in the rate of growth in leaflet C occurred on May 17, with a drop from 22 per cent to an average of 5 per cent. In leaflet D the more rapid rate of growth continued for a

slightly longer period, until May 20, when the rate of growth fell from 10 per cent to an average of 5 per cent for the remaining period of observation.

The curves of leaflet and of leaf growth (fig. 2) conform to the typical curve expressing the rate and distribution of growth in any of the other plant organs, such as stem or root. These curves of percentage increase were obtained by using the increase as the numerator and the area of the preceding day as the denominator of a

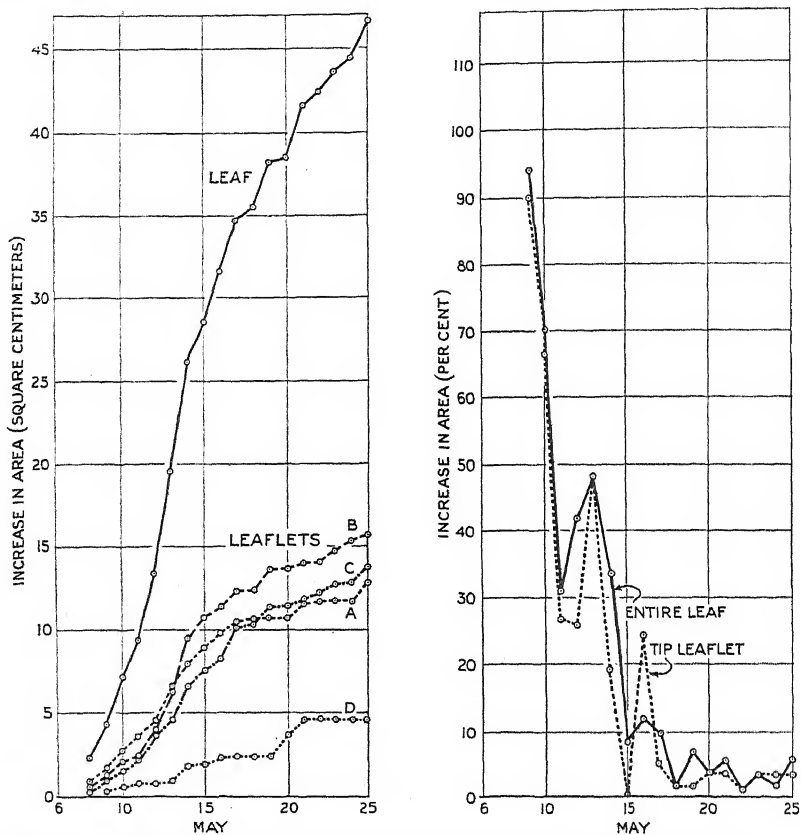


FIGURE 2.—Increase in area of entire leaf, and the four leaflets A to D, respectively, shown in Figure 1, and the percentage increase in the entire leaf and the tip leaflet

fraction. If the leaf was small and the increase in length and width was 1 mm, the percentage of growth was very much greater than if the leaf was much larger in area. Such a curve represents the proportionate growth more fairly than does a curve where the increment is simply added to a previous area. Such curves are given for the tip leaflet and for the entire leaf.

#### GROWTH OF LEAVES ON A SINGLE SHOOT

All leaves on three plants, each consisting of a single shoot, were measured between May 8 and May 25, 1931, in the manner described. Leaf 1 was nearest the base of the plant. By the end of the observa-



tion period one cluster of flower buds had appeared and a second had begun to form in the axil of one of the branches, the plant being then about 30 cm high.

The curves of growth of the leaves of the largest and sturdiest of the plants, as shown in Figure 3, indicate that while the basal leaf

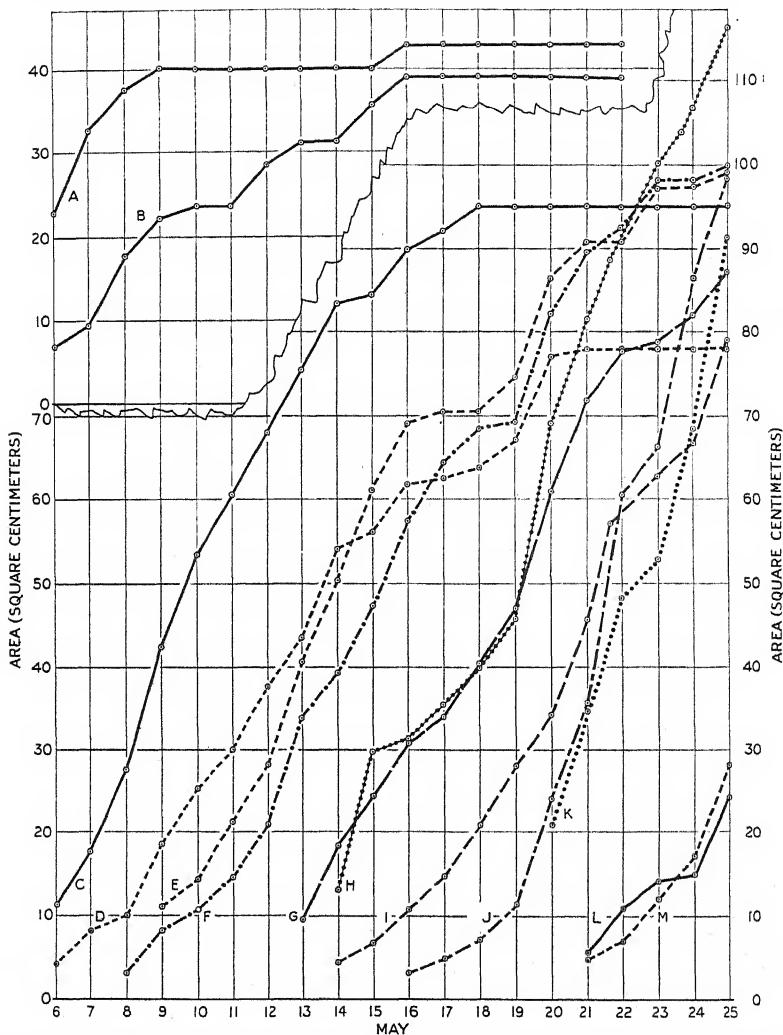


FIGURE 3.—Growth of all leaves on a shoot, leaf A being nearest the ground. Accelerated growth shifts to the right, i. e., up the stem, as new leaves are formed

grew very rapidly during the early days of the observations, this increase dropped off until its growth practically ceased. The period of accelerated growth on leaf 2 came a few days later, and growth continued over a longer period, but gradually it too came to a standstill. The growth of leaf 3 moved over still further toward the later period before growth slowed down, and the same was true of leaves 4, 5, and 6. Leaves 7 to 13 were still in the stage of rapid growth

when the observations were concluded on May 25. In fact, these leaves were so small that measurements could not be made on leaf 7 until May 13, and the first measurements on leaves 12 and 13 were not taken until May 21.

The five to seven leaflets which make up the compound leaf of the potato show almost simultaneously accelerated growth, although, as

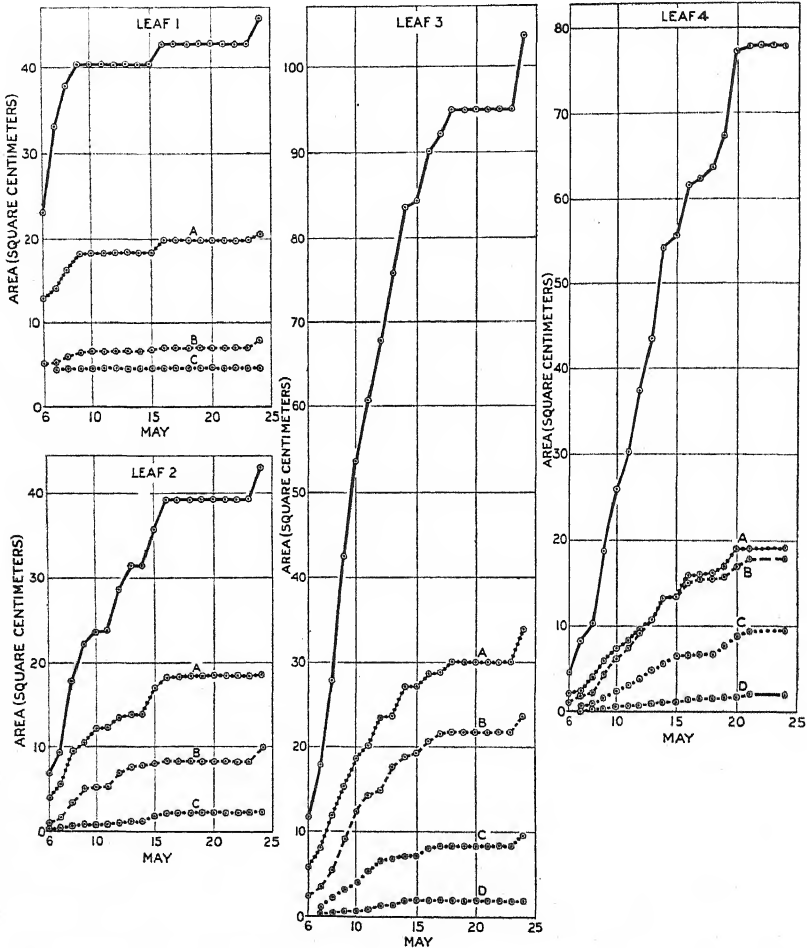


FIGURE 4.—Growth of representative leaves (solid lines) and their respective leaflets A-C and A-D

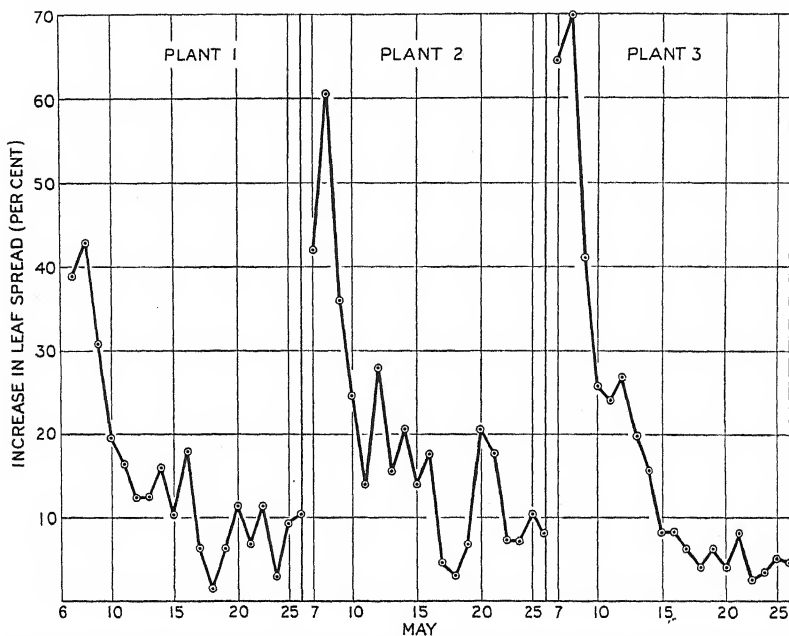
noted above, the period of most rapid growth moves slightly forward in point of time from the tip leaflet toward the base. (Fig. 4.)

The percentage of increase in the total leaf spread of three plants (fig. 5) indicates that, for a time after the plants are well above the surface of the ground, the rate of growth is very rapid. Then for a few days the increase is only 10 to 20 per cent each day, and thereafter it falls to an average of less than 10 per cent a day.

Almost identical curves were obtained from outdoor growth of leaves between July 17 and August 1. Some of the very small leaves

at the base of the plants were not measured. The leaves on the outdoor plants were larger than those on the greenhouse plants, but the slowing up of growth in older leaves and the beginning of growth in younger ones during the latter part of the observations were similar in both, although the outside temperature was never quite so high during the day as that in the greenhouse and several times at night sank below the minimum of the greenhouse.

Increase in leaf spread was continuous during the observations, as is indicated by the number of square centimeters of leaf. Flower



extent before they divide, but real increase in bulk comes after they start to grow by formation of vacuoles in the cytoplasm.

The leaflets selected showed variations in growth. (Table 1 and fig. 6.) In the greenhouse plants growth was fairly evenly distributed

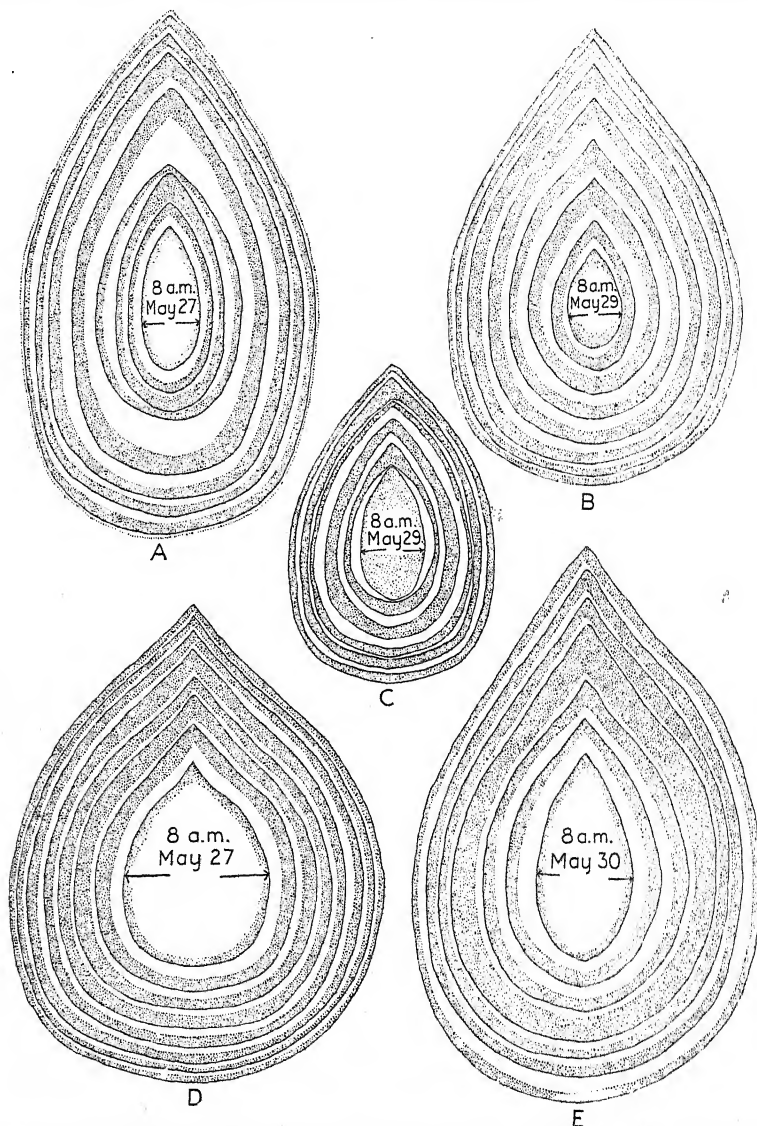


FIGURE 6.—Growth during day and night of five typical leaflets on plants in the greenhouse; daylight growth in white, night growth shaded

between day and night, although the night growth was a little greater. In certain leaflets the growth was quite regular; in others it was rather irregular. Occasionally it was greater at night, but frequently it was greater during the day. (Fig. 7.) No adequate explanation of this difference in the growth of individual leaves can be offered.

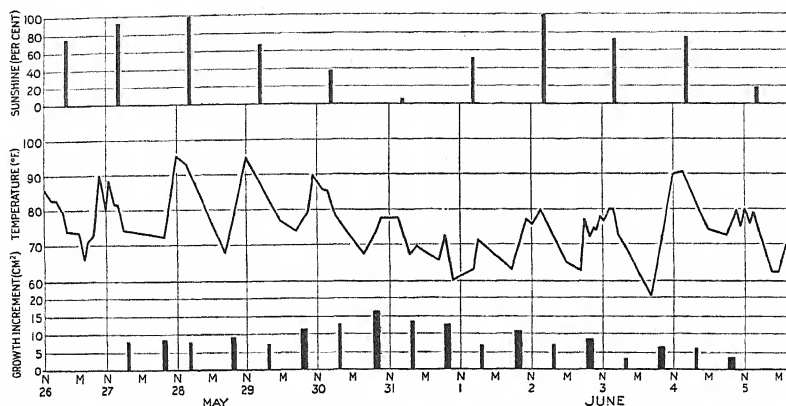


FIGURE 7.—Correlation of growth increments of 10 leaflets on greenhouse plants with temperatures and percentage of sunshine distributed about noon (N) and midnight (M). Night growths are shown by heavy and day growths by light black bars

TABLE 1.—Area increase in square centimeters of three leaves on greenhouse plants by day and by night

Leaflet and date	Leaf 1		Leaf 2		Leaf 3	
	Day	Night	Day	Night	Day	Night
<b>Leaflet A:</b>						
May 27.....	0.64	0.73	0.65	0.84	1.37	0.95
May 28.....	.67	.96	1.00	1.43	1.03	.84
May 29.....	.88	.37	1.34	1.83	1.09	1.20
May 30.....	1.59	1.27	1.93	3.05	1.91	2.00
May 31.....	.85	.90	.87	2.13	1.77	1.63
June 1.....	.64	.87	2.34	1.56	1.28	.87
June 2.....	.90	.73	.73	3.19	2.00	1.56
Total.....	6.17	5.83	8.86	14.03	10.45	9.05
<b>Leaflet B:</b>						
May 27.....	.26	.11	.33	.30	.17	.10
May 28.....	.15	.38	.35	.35	.37	.43
May 29.....	.28	.23	1.01	1.14	.36	.26
May 30.....	1.04	.57	.84	1.24	1.11	.87
May 31.....	.87	.89	1.45	1.49	.60	.67
June 1.....	.34	.59	.....	.....	.61	1.41
June 2.....	.34	.96	.....	.....	.05	.43
Total.....	3.28	3.73	3.98	4.58	3.27	4.17
<b>Leaflet C:</b>						
May 27.....	.14	.18	.15	.21	.15	.40
May 28.....	.26	.35	.17	.47	.37	.43
May 29.....	.52	.16	.49	.44	.52	.37
May 30.....	.30	.19	.47	.96	.72	.60
May 31.....	.39	.41	.35	.39	.42	.86
June 1.....	.....	.....	.39	.56	.23	.72
June 2.....	.....	.....	.....	.....	.37	.25
Total.....	1.51	1.29	2.02	3.03	2.78	3.63

The percentage of night growth of three leaves from greenhouse plants follows:

Leaf 1:	Per cent	Leaf 2:	Per cent	Leaf 3:	Per cent
Leaflet A.....	48.6	Leaflet A.....	61.3	Leaflet A.....	46.4
Leaflet B.....	53.2	Leaflet B.....	53.5	Leaflet B.....	56.0
Leaflet C.....	46.1	Leaflet C.....	60.0	Leaflet C.....	56.6
Average.....	49.3	Average.....	58.3	Average.....	53.0

Average night growth, nine leaflets, 53.5 per cent.

Average day growth of same leaflets, 46.5 per cent.

The average growth of 10 leaflets from three different plants during the same 8-day period was as follows: Day growth, 135 cm<sup>2</sup>, or 45.9 per cent;<sup>3</sup> night growth 159 cm<sup>2</sup>, or 54.1 per cent.

Night growth was slightly greater than day growth in spite of the fact that night temperatures were much lower than day temperatures. In the greenhouse the temperature was as low as 60° F. at night, whereas during the day it sometimes rose above 100° in full sunlight.

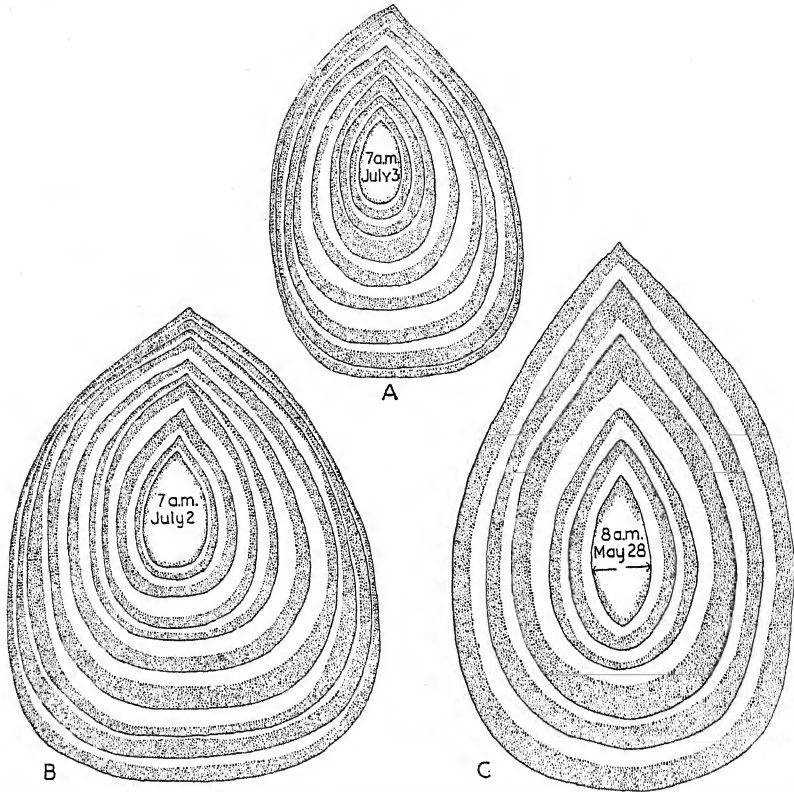


FIGURE 8.—Growth during day and night of three typical leaflets on plants grown out of doors; day-light growth in white, night growth shaded

The thermograph was shaded and so did not record the maximum temperature in full sunlight.

The relation of day and night growth to sunshine and temperature is shown in Figure 7. The sunshine records were obtained from the near-by weather bureau station at Burlington, Vt.; the temperature records were taken with a Friez recording thermograph. The period of maximum growth was from May 30 to June 2, the peak occurring on May 31. This period coincides with that of minimum sunshine and low temperatures both day and night. The days preceding this period had been almost cloudless and the temperature high both day and night, but the effect of these conditions was not shown by the

<sup>3</sup> Cm<sup>2</sup> is the abbreviation for square centimeter recently adopted by the Style Manual for United States Government printing.

leaves until a few days later. Apparently these leaflets had reached the period of maximum development, and the process continued in spite of slightly unfavorable surroundings, or perhaps the very favorable conditions for carbohydrate formation did not show in the plant until several days after they had passed. Fischer (5) in a study of the growth of rye plants found that favorable or unfavorable effects of temperature and moisture were not immediately apparent but were expressed by the plant in the period that followed and the stimulus of a favorable or unfavorable period varied directly as its length.

#### DAY AND NIGHT GROWTH OF LEAVES ON OUTDOOR PLANTS

Three leaves, each with a terminal leaflet and two pairs of side leaflets, were measured at 7 a. m. and at 7 p. m. At the beginning of the measurements the leaves were just entering the period of rapid growth. The records were kept as long as the measurements showed differences in day and night growth. The time selected, July 21 to 27, is the period of most rapid foliage growth in the field in this locality. The results of the measurements are given in Table 2. Figure 8 shows day and night growth in three typical leaflets chosen from supplementary series of measurements. In these leaflets also night growth was slightly in excess of day growth.

TABLE 2.—Area increase in square centimeters of three leaves on outdoor plants, by day and by night

Leaflet and date	Leaf 1		Leaf 2		Leaf 3	
	Day	Night	Day	Night	Day	Night
Leaflet A:						
July 21.....	1.64	3.63	0.52	4.74	1.73	3.62
July 22.....	1.94	3.82	3.11	3.00	4.00	3.18
July 23.....	0	4.51	.72	3.54	4.10	5.77
July 24.....	2.48	4.97	1.34	2.68	1.32	2.46
July 25.....	2.94	4.80	5.73	2.51	6.21	2.39
July 26.....	4.52	1.80	5.24	4.41	4.78	3.79
July 27.....	4.40	0	0	3.31	0	2.95
Total.....	17.92	23.53	16.66	24.19	22.14	24.16
Leaflet B:						
July 21.....	.52	.73	.43	1.56	.92	.62
July 22.....	1.08	1.04	.46	1.27	1.17	.84
July 23.....	1.27	.78	.71	.72	2.39	2.49
July 24.....	1.87	2.50	1.20	1.96	.75	1.48
July 25.....	1.54	4.10	1.81	1.57	2.76	2.91
July 26.....	2.66	3.25	1.97	2.12	4.97	2.52
July 27.....	.48	2.23	2.09	.62	0	3.38
Total.....	9.42	14.63	8.67	9.82	12.96	14.24
Leaflet C:						
July 21.....						
July 22.....	.49	0	.71	.23	.63	1.08
July 23.....	1.34	.58	.53	.96	.94	.66
July 24.....	.76	1.17	.50	2.04	1.37	1.45
July 25.....	.98	1.47	1.89	2.55	.76	2.42
July 26.....	1.08	1.51	2.51	1.70	2.32	1.81
July 27.....	1.57	.43	1.70	.72	.50	.88
Total.....	6.22	5.16	7.84	8.29	6.52	8.30

The percentage of night growth of three leaves from plants grown out of doors is as follows:

Leaf 1:	Per cent	Leaf 2:	Per cent	Leaf 3:	Per cent
Leaflet A.....	56.7	Leaflet A.....	59.2	Leaflet A.....	52.2
Leaflet B.....	60.8	Leaflet B.....	53.1	Leaflet B.....	52.3
Leaflet C.....	45.3	Leaflet C.....	51.4	Leaflet C.....	56.0
Average.....	54.3	Average.....	54.6	Average.....	53.5

Average night growth, nine leaflets, 54.1 per cent.

Average day growth of same leaflets, 45.9 per cent.

The average growth of 10 leaflets from three outdoor plants during the same 8-day period was as follows: Day growth, 167 cm<sup>2</sup>, or 43.2 per cent; night growth, 220 cm<sup>2</sup>, or 56.8 per cent.

The relative independence of growth and temperature is further emphasized by Figure 9. For example, the night temperatures of July 22-23, July 24-25, and particularly of July 25-26 were very low, but on these dates night growth was very rapid, and on the last-

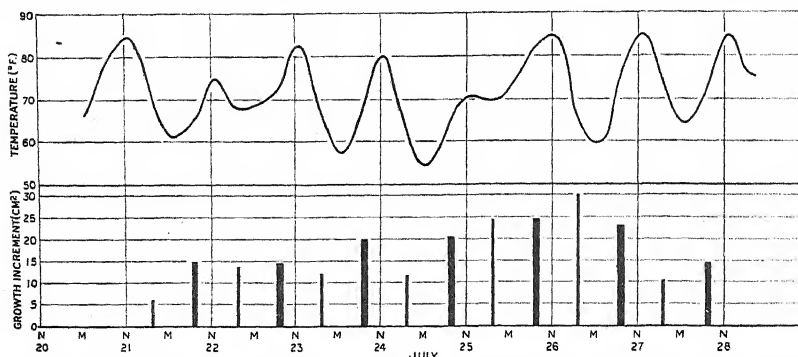


FIGURE 9.—Correlation of growth increment of 10 leaflets on plants out of doors, with daily temperatures distributed about noon (N) and midnight (M). Night growths shown by wide and day growths by narrow black bars

named date it was greater than for any other night period. Apparently growth, once started, proceeds almost in complete disregard, for a time at least, of the temperature of the environment.

As in leaflets on greenhouse plants, great irregularity occurred in the day and night growth of individual leaves and leaflets. Apparently internal factors are more important than external ones, at least in their immediate effects.

#### DISCUSSION

Decrease in the growth rate of each succeeding leaf is conditioned on the formation of a new leaf which removes part of the food supply. As soon as a new leaf is formed, the growth of the one below it is checked and the new leaf takes on accelerated growth. During the period of vegetative growth the wave of acceleration passes up the stem from one leaf to the next above it. In the potato this growth movement is not checked by the development of flowers, as very little material passes into them and they rarely set seed.

In the embryonal condition the leaf grows slowly, as the material for tissue formation must be drawn largely from older leaves through the stem, which serves as a common reservoir for food materials. As



soon as the young leaves are established, however, they constantly use more food, partly of their own manufacture and partly that made by the older leaves. Thus, the new leaves slow down the growth of the older ones. Some general factor must also be involved, probably that of permeability; the older membranes being less easily penetrated by solutions than the younger ones, the current of food takes the path of least resistance, i. e., into the younger organs.

### SUMMARY

All leaves on a single potato shoot were measured at intervals for a period of about 20 days in the greenhouse during May and in the field in July.

Growth of leaflets and leaves followed the typical S-shaped curve. The leaves first formed stopped growth during the early part of the observations, and the next younger leaves then began rapid growth. At the close of the observations the youngest leaves were just beginning this period of accelerated growth.

The rate of growth of leaflets composing a single leaf is not always the same on any single day; so growth seems to be more or less independent of environment.

The growth of a shoot continues after the flower clusters are formed, a new vegetative shoot pushing them to one side. In this manner, the shoot continues to grow and the foliage to increase in spread.

Measurements on rapidly growing leaflets taken in the morning and at night showed: In greenhouse plants, night growth, 54.1 per cent; day growth, 45.9 per cent; in outdoor plants, night growth, 56.8 per cent; day growth, 43.2 per cent.

The accelerated growth of each leaf seems to be checked by the development of a newer leaf. The checking of vegetative growth in the potato plant, however, is not due to flower formation, for growth continues after flower clusters have appeared.

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## HIBERNATION OF THE MEXICAN BEAN BEETLE IN THE ESTANCIA VALLEY, N. MEX.<sup>1</sup>

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### INTRODUCTION

The investigation described in this paper formed part of a comprehensive study of the hibernation of the Mexican bean beetle (*Epilachna corrupta* Muls.) made in the Estancia Valley, N. Mex., from 1923 to 1929. The investigation was undertaken to determine, if possible, the relative effects of hibernation material, snowfall coverage, temperature, precipitation, and climatic fluctuation on the overwintering of the beetle. In addition, information was sought which would be useful in determining the possible economic importance of this insect in noninfested bean-growing areas in advance of its spread to these areas.

Repeated observations have shown that the Mexican bean beetle is a major pest of beans only when they are grown near suitable hibernation quarters. In the Estancia Valley beetles begin their fall migration in search of hibernation quarters early in September and reach their maximum flight during the latter part of September or the first part of October, depending on the seasonal variation in weather conditions, especially the occurrence of killing frost, and on the time the bean plants are harvested. The beetles begin to leave their winter quarters and appear in the fields early in June. The peak of the infestation of overwintered beetles occurs in the foothill fields during July, the exact time depending on the quantity of rainfall. In this paper winter survival and overwintering refer to the entire hibernation period.

### METHODS OF INVESTIGATION

In these studies 216,340 beetles were used. They were collected from the following places: In 1923-24, from irrigated gardens in the Estancia Valley; in 1924-25, from the foothills of the Estancia Valley and the Rio Grande Valley, between Belen and Los Lunas, N. Mex.; in 1925-26 and 1926-27, from irrigated bean fields near Hoehne, Colo., and in the Rio Grande Valley north of Albuquerque, N. Mex.;

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in 1927-28 and 1928-29, from the Rio Grande Valley, near Albuquerque and Belen.

In making the collections the plants were jarred with a thin board and the beetles fell into a specially constructed pan. After several hundred beetles had been collected, they were emptied into cloth sacks which the collectors carried in their belts. At intervals the contents of the sacks were dumped into a cage, in which they were transported to the laboratory by autotruck. At the laboratory the beetles were fed twice daily, the old refuse being removed from the cage at the time of feeding. Wet gunny sacks were placed over the cages to reduce the temperature and increase the humidity. The beetles were counted in the order of collection, so that each group was held at the laboratory approximately the same length of time. They were first placed in wide-mouth bottles and chilled in ice water to reduce their activity and permit rapid and accurate counting. When the desired number of beetles had been counted, they were placed in cylindrical carrying cases containing bean foliage and transferred to the hibernation cages in the field at a time when field beetles were seeking hibernation quarters.

During the season of emergence from hibernation, the cages in the foothills and valleys were observed as often as time and conditions would allow, generally daily or at least every other day. The cages located on the mountain were observed less frequently. After emergence had become general, the active beetles were removed from the cages on days when examinations were made and the numbers thus removed were recorded as the ones that had survived. These numbers were used in computing the percentages given in Table 2.

#### DESCRIPTION OF CAGES

The hibernation cages were constructed of 2 by 4 inch lumber, and measured 4 feet wide, 6 feet long, and 2½ to 4 feet high. They were covered with 14-mesh screen wire and had removable tops. (Fig. 1.) After the beetles had become dormant, the tops were removed and the cages covered with 1-inch mesh wire. This permitted snow to enter the cages but excluded intruders. The original tops were replaced in the spring before the beetles resumed their activity. In this manner conditions closely approximating those prevailing in the surrounding area were secured. Extra heavy wire screening was placed vertically in the soil to a depth of 12 inches around the base of each cage to prevent burrowing animals from gaining access to the hibernating beetles. When the cages were placed in the soil, care was taken to preserve the forest floor or natural cover.

#### LOCATION OF CAGES

Details as to the location of the hibernation cages are given in Table 1. Cage 1 was located near the upper edge of the fir-spruce association, or Canadian Zone, and cages 2 and 3 near the lower border of this zone. Cage 4 was placed at the upper edge of the ponderosa (western yellow) pine forest, or Transition, zone, and cages 5, 6, 7, and 8 in the lower half of this zone. Cage 9 was located in the piñon or nut-pine association that clothes the lower rolling foothills bordering the valley on the west and the ponderosa pine forest zone. Cages 10, 11, and 12 were placed in the short-grass or semidesert formation

of the Estancia Valley. The line of hibernation cages extended in an eastern and western direction for 25 miles and ascended from an elevation of 6,100 to one of 9,000 feet.

Cage 2 was placed against the base of a high rim rock, which afforded some protection and decreased the amount of precipitation entering the cage. Cages 5 and 7 were located on the south side of Tajique Canyon just below the first rim rock and were also well protected.



FIGURE 1.—Type of cage used in hibernation investigation, with instrument shelter in background .

Cage 8 was placed at the base of a steep hill, 30 feet from the canyon stream, where it received a large amount of moisture from both precipitation and seepage. Cage 11 was set between the laboratory and the insectary, where it received the maximum quantity of snow and, being protected from the wind, the hibernation material was evenly covered. The remaining cages were not protected from the wind and in them the snow coverage was not evenly distributed over the hibernation material.

#### WEATHER RECORDS

The temperature, precipitation, and snowfall records have been compiled from the meteorological records of cooperative observers of the United States Weather Bureau and from records taken at the Estancia laboratory. The three cooperating weather stations are located as follows: (1) At Rea's ranch, on Bosque Mountain, at an elevation of 9,215 feet, latitude  $34^{\circ} 46' N.$ , longitude  $106^{\circ} 20' W.$ , in the Canadian Zone, near cages 1 to 4, inclusive; (2) near Tajique, in the foothills at an elevation of 7,100 feet, latitude  $34^{\circ} 48' N.$ , longitude  $106^{\circ} 18' W.$ , 3 miles northeast of the foothill cages in the Transition Zone; (3) at Estancia, in the Upper Sonoran Zone, at an elevation of 6,100 feet, latitude  $34^{\circ} 45' 20'' N.$ , longitude  $106^{\circ} 3' W.$ , in the short-grass or semidesert formation, near cages 10, 11, and 12.

TABLE 1.—Location of hibernation cages

## CANADIAN ZONE

Cage No.	Situation	Slope of situation	Elevation	Exposure	Topography	Soil	Environment	Plant association
		Degrees	Feet					
1	Mountain	45	9,000	North	Mountain side	Black loam	Dense forest of fir and spruce, a few maple and black locust.	Fir-spruce.
2	do	45	8,400	do	Midway on slope of mountain canyon.	do	Dense forest of scrub oak, maple, and a few fir and spruce.	Do.
3	do	45	8,400	do	do	do	do	Do.

## TRANSITION ZONE

4	Mountain	10	8,400	Southeast	Mountain side	Black loam	Dense forest of scrub oak, a few pine, cedar, and black locust.	Pine.
5	Foothill	20	7,050	North	On side of broad canyon.	do	Open forest of pine and white oak, dense near cage.	Do.
6	do	20	7,050	East	Hill, near center of slope	Sandy black loam	Open forest of pine and white oak, large pines near cage.	Do.
7	do	7.5	7,000	do	On side of broad canyon	Black loam	Open forest of pine and white oak	Do.
8	do	45	6,975	North	Near bottom of narrow canyon.	do	Open forest of pine and white oak, dense near cage.	Do.

## UPPER SONORAN ZONE

9	Foothill	2	6,500	North	Hill, near top	Red clay loam	Open forest of cedar and piñon.	Piñon-juniper.
10	Valley	0	6,100	Open	Broad valley	Sandy gray loam	Sod	Short grass, or semidesert.
11	do	0	6,100	do	do	Black loam	Transplanting bed near insectary and laboratory	Do.
12	do	0	6,100	do	do	Sandy gray loam	Sod	Do.

## GENERAL FEATURES OF ESTANCIA VALLEY

## PHYSIOGRAPHY

Estancia Valley<sup>3</sup> (fig. 2) extends from about latitude  $34^{\circ} 20'$  to  $35^{\circ} 17' N.$ , and longitude  $105^{\circ} 42'$  to  $106^{\circ} 24' 30'' W.$  Its drainage basin forms a depression with no outlet, having a maximum extent of about 65 miles north and south and 40 miles east and west, and includes an area of about 2,000 square miles.

Four physiographic areas are recognized in the valley—the valley floor, hills, mesas, and mountains. Estancia Valley is separated from the Rio Grande Valley by the Manzano Mountain range, which extends north and south and parallels the two valleys for 30 miles as an unbroken mountain wall. This range has an abrupt slope fronting

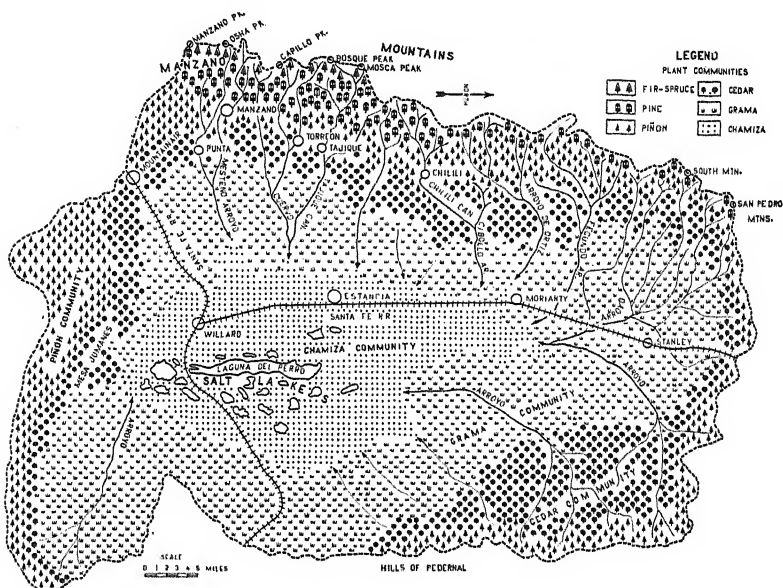


FIGURE 2.—Sketch of Estancia Valley, showing plant communities

the Rio Grande Valley by reason of an uplift which exposes the rock strata on its western slope in the form of a precipitous escarpment of about 4,000 feet. The long eastern slope follows the dip of these strata and gradually descends into the foothills of Estancia Valley. (Fig. 3.) At the northwest corner of the valley are South Mountain and the San Pedro Mountains, two isolated masses. Between South Mountain and the north end of the Manzano Range, a distance of nearly 15 miles, the mountain wall is interrupted, the divide between the Estancia and Rio Grande Valleys here being formed by a rugged hilly tract. From the center of the valley northward the surface rises gently to a point where the plain ends abruptly in an escarpment. On the northeast the valley is bordered by a mesa. Farther south are the Hills of Pederal, which divide the valley from the treeless grass-

<sup>3</sup> MEINZER, O. E. GEOLOGY AND WATER RESOURCES OF ESTANCIA VALLEY, NEW MEXICO, WITH NOTES ON GROUND-WATER CONDITIONS IN ADJACENT PARTS OF CENTRAL NEW MEXICO. U. S. Geol. Survey Water-Supply Paper 275, 89 p., illus. 1911.

land plains of the Pecos slope. The hills that inclose the valley on the southwest are lower and less rugged. On the southeast the valley is terminated abruptly by the Mesa Jumanes, whose escarpment, 500 feet high, forms an imposing physiographic feature. The area drains into a series of large salt lakes in the southeastern part of the valley.

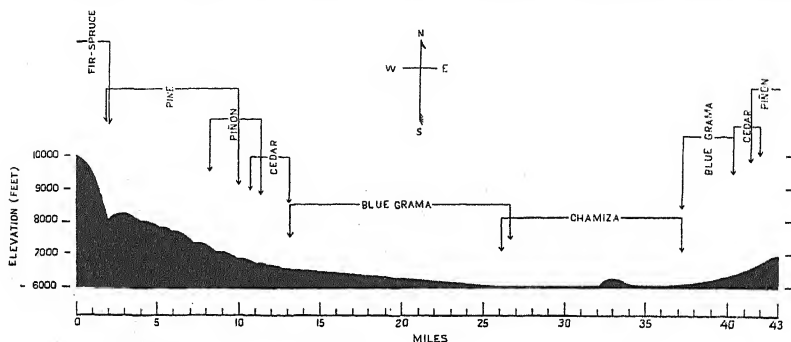


FIGURE 3.—Cross section of the plant associations in the Estancia Valley

The lowest point in the valley has an elevation of about 6,000 feet and the highest point in the Manzano Mountains is about 10,000 feet.

The climate of this area, with its cool summers and cold winters, is determined by its elevation, its inclosed nature, and the altitudinal range of approximately 4,000 feet. The highest and lowest temperatures occur at the lowest elevations.

#### PLANT ASSOCIATIONS

The valley floor is divided into two plant zones, the alluvial slopes or plains and the ancient lake-bed region. The alluvial slopes are covered with a short-grass sod in which blue grama (*Bouteloua gracilis*)



FIGURE 4.—Blue grama (*Bouteloua gracilis*) sod

(fig. 4) predominates. The lake-bed area is dominated by a small-leaved shrub, fourwing saltbush (*Atriplex canescens*), commonly called chamiza in New Mexico. (Fig. 5.) In the fourwing-saltbush com-



munity are extensive areas that have a shallow water table and a high alkali content. These areas are covered by bluestem (*Andropogon furcatus*). Although most of the central part of the valley is flat,



FIGURE 5.—Fourwing saltbush (*Atriplex canescens*) that is found on the extensive areas of the old lake and the salty mud flats

extensive depressions containing salty mud flats (salt basins) devoid of vegetation are found.

The western foothills are divided into four forest zones (fig. 2), whereas the other sides of the valley afford only two. The lower roll-

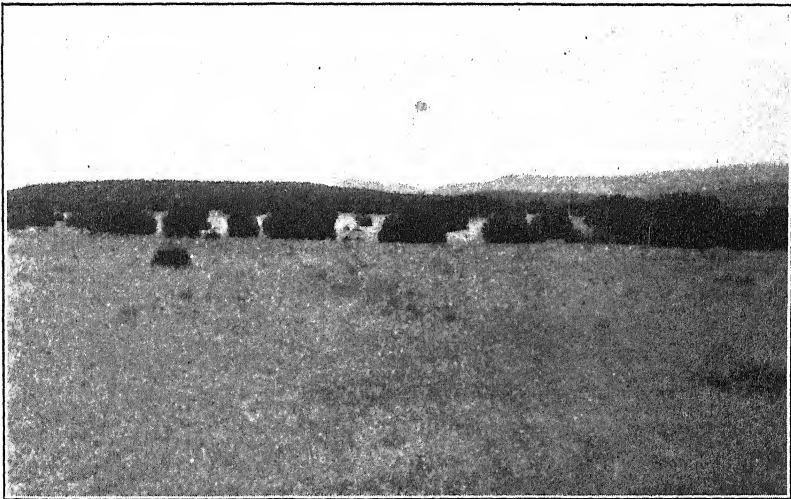


FIGURE 6.—Cherrystone juniper (*Juniperus monosperma*) that sparsely clothes the lower rolling hills

ing hills that border the valley on the east and west are sparsely clothed with cherrystone juniper (*Juniperus monosperma*). (Fig. 6.) The mesa and the intermediate forest or nut-pine region are covered

with piñon (*Pinus edulis*). (Fig. 7.) (These types of vegetation fall within the Upper Sonoran Life Zone of Merriam.) The ponderosa pine (*Pinus ponderosa*) covers the higher rolling hills (Transition Zone)

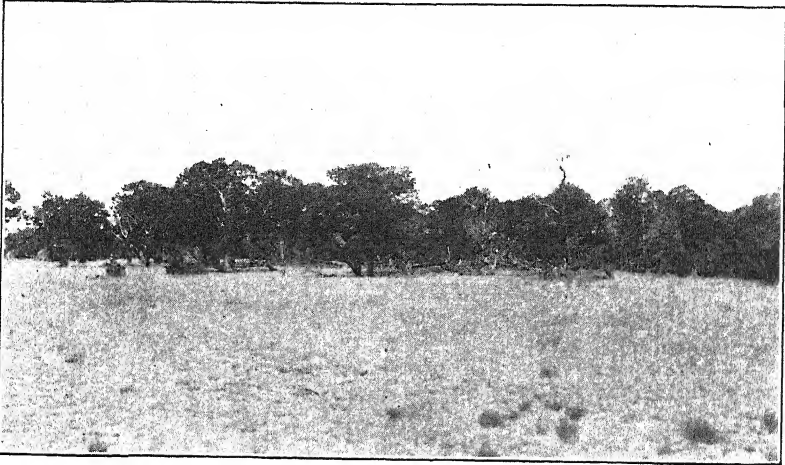


FIGURE 7.—Piñon (*Pinus edulis*) that covers the mesa and intermediate forest or nut-pine region

along the foot of the Manzano Mountains and the lower and drier slopes of the mountains. (Fig. 8.) In places there are relatively open stands of this pine with a clean floor, but more often there is an under-



FIGURE 8.—Ponderosa pine (*Pinus ponderosa*) that covers the higher rolling hills and the lower and drier slopes of the mountains

growth of trees and shrubs, the most common being the oak (*Quercus gambelii*). In the elevated areas of the Manzano Mountains (Canadian Zone) the dominant evergreen trees are Douglas fir (*Pseudotsuga mucronata*) and Engelmann spruce (*Picea engelmanni*). White oaks

(*Quercus utahensis* and *Q. novomexicana*), Rocky Mountain maple (*Acer glabrum*), and quaking aspen (*Populus tremuloides*) are the deciduous trees occurring in greatest abundance.

## HIBERNATION OF BEETLES IN THE VARIOUS LIFE ZONES

### CANADIAN ZONE

The results obtained in the Canadian Zone in a period of five years show that in four of the years no beetles survived and in the fifth year (1926-27) only 42, or 0.56 per cent, survived. (Table 2.) During the 5-year period a total of 38,500 beetles was used, and during 1926-27, 7,500 beetles were used; the percentage survival for the five years was therefore 0.11. The winter of 1926-27 was unusually warm, with an average daily departure from normal temperature of 2° F. The precipitation was nearly normal.

In order to determine whether the character of the hibernation material was an important factor in the survival of beetles in this zone, pine needles were added to the oak leaves in cages 2 and 3 in the fall of 1926 and in the following years. (Table 2.) In 1927-28, when the average daily temperatures was 0.9° F. above normal and the precipitation 0.21 inch below normal (Table 9), no beetles survived. In 1928-29 the departures from normal were -1.4° F. and +0.26 inch of precipitation, and again no beetles survived. It is evident that in this zone winter mortality is due to climatic factors, and not to the character of the material used as a shelter for the beetles.

### TRANSITION ZONE

In the Transition Zone 133,340 beetles were used in six years, with a total survival of 13,114, or 9.84 per cent. At the upper edge of the ponderosa pine belt (cage 4) 5.01 per cent survived over a period of six years. In cages 5 and 7 more than 12 per cent survived the dormant period. The locations of these cages are typical of favorable natural hibernation quarters. Cage 8 showed the lowest percentage of survival in this zone, 3.16 per cent. It is doubtful whether beetles in search of hibernation quarters would under natural conditions attempt to hibernate in locations in which moisture conditions were so unfavorable as they were in this cage.

The results of the six years' experiments indicate that the ponderosa pine forest zone (fig. 8) is the natural hibernation quarters of the beetle in the West and that it is even more favorable when oak trees are present in the association. This indication is confirmed by the fact that beetles are found hibernating naturally chiefly in this zone.

### UPPER SONORAN ZONE

In the Upper Sonoran Zone 44,500 beetles were employed with a total survival of 1,210, or 2.72 per cent, in all four cages (Nos. 9 to 12) where materials both native and foreign to the zone were used as shelter. In cases 9 and 10, where the sheltering material was native to the zone, the percentage of beetles which hibernated successfully averaged 0.34. This average does not include the results for cage 9 in the season 1926-27, when the compact piñon needles in that cage were loosened in order to test the suitability of masses of needles as hibernation material. In the loosened needles 16.68 per cent of the insects

TABLE 2.—Hibernation results, 1923-24 to 1928-29

## CANADIAN ZONE

Cage No.	Season	Hibernation material	Date beetles were introduced	Beetles introduced	Beetles survived	
				Number	Number	Per cent
1	1924-25	Spruce needles and cones	Sept. 30	5,000	0	0
	1925-26	Maple leaves and spruce needles	Oct. 10	2,500	0	0
	1926-27	do.	Sept. 29	2,500	2	.08
	1927-28	do.	Oct. 4	1,000	0	0
	1928-29	do.	Sept. 28	2,500	0	0
2	1924-25	Oak leaves and fir needles	Oct. 2	3,000	0	0
	1925-26	do.	Oct. 8, 15	5,000	0	0
	1926-27	Oak leaves and pine needles	Sept. 29	2,500	28	1.12
	1927-28	do.	Oct. 4	1,000	0	0
	1928-29	do.	Sept. 28	2,500	0	0
3	1925-26	Oak leaves and fir needles	Oct. 8, 15	5,000	0	0
	1926-27	Oak leaves and pine needles	Sept. 29	2,500	12	.48
	1927-28	do.	Oct. 4	1,000	0	0
	1928-29	do.	Sept. 28	2,500	0	0

## TRANSITION ZONE

4	1923-24	Oak leaves and pine needles	Oct. 9	5,004	208	4.16
	1924-25	Oak leaves and cedar needles	Oct. 7	2,000	9	.45
	1925-26	do.	Oct. 15	1,500	36	2.40
	1926-27	do.	Sept. 29	1,500	248	16.53
	1927-28	do.	Oct. 4	1,000	3	.30
5	1928-29	do.	Sept. 28	2,500	172	6.88
	1924-25	Oak leaves and pine needles	Oct. 15	1,296	130	10.03
	1925-26	do.	Oct. 10, 12	5,000	1,112	22.24
	1926-27	do.	Oct. 2	10,000	2,829	28.29
	1927-28	do.	Oct. 3, 5	10,000	75	.75
6	1928-29	do.	Sept. 29	10,000	295	2.95
	1925-26	Pine needles	Oct. 19	1,500	339	22.60
	1926-27	do.	Oct. 4	1,500	<sup>a</sup> 206	13.73
	1927-28	do.	Oct. 6	2,500	<sup>b</sup> 123	4.92
	1928-29	do.	Oct. 4	2,500	<sup>b</sup> 38	1.52
7	1923-24	Oak leaves and pine needles	Oct. 17	5,540	1,304	23.54
	1924-25	do.	Oct. 5, 7	5,000	363	7.26
	1925-26	do.	Oct. 11	5,000	1,812	36.24
	1926-27	do.	Oct. 3, 9	15,000	2,433	16.22
	1927-28	do.	Oct. 3, 5	10,000	230	2.30
8	1928-29	do.	Oct. 4	10,000	358	3.58
	1925-26	do.	Oct. 10 to 20	10,000	183	1.83
	1926-27	do.	Oct. 4	5,000	516	10.32
	1927-28	do.	Oct. 5	5,000	90	1.80
	1928-29	do.	Oct. 4	5,000	2	.04

## UPPER SONORAN ZONE

9	1925-26	Pifion needles	Oct. 11	2,500	70	2.80
	1926-27	Pifion needles (loosened material)	Oct. 4	2,500	417	16.68
	1927-28	Pifion needles	Oct. 6	2,500	0	0
	1928-29	do.	Oct. 4	2,500	0	0
	1923-24	Russian thistles and other weeds	Oct. 23	2,500	4	.16
10	1924-25	Bean hulls and Russian thistles	Oct. 11	2,000	4	0
	1925-26	do.	Oct. 10	2,500	0	0
	1926-27	do.	Oct. 7	2,500	0	0
	1927-28	do.	do.	2,500	0	0
	1928-29	do.	Oct. 10	2,500	0	0
11	1925-26	Oak leaves and pine needles	Oct. 18	2,500	545	21.80
	1926-27	do.	Oct. 7	5,000	103	2.06
	1927-28	do.	do.	2,500	23	.92
	1928-29	do.	Oct. 4	2,500	28	1.12
	1926-27	do.	Oct. 8	2,500	20	.80
12	1927-28	do.	Oct. 7	2,500	0	0
	1928-29	do.	Oct. 4	2,500	0	0

<sup>a</sup> On May 3 shelter placed over cage to exclude natural precipitation.<sup>b</sup> On May 10 shelter placed over cage to exclude natural precipitation.

passed the winter successfully. In the three seasons that cage 9 contained compact masses of needles, not a single beetle survived during two seasons and only 2.8 per cent during another season, the average survival for the three seasons being 0.93 per cent. These results indicate that the natural hibernating material in the cherry-stone juniper and piñon forest zone does not provide proper conditions for winter survival of the beetle, owing primarily to the fact that few of the beetles are able to enter the compact masses of needles. This conclusion conforms with observations made by Graf,<sup>4</sup> who did not find a single beetle hibernating in the piñon pine and cherrystone juniper needles in the foothills during the season of 1921-22, when the heaviest infestation of beetles ever known in the Estancia Valley was recorded. The needles of the piñon and cherrystone juniper trees are very short, and only a light fall occurs yearly, resulting in a scant compact mass around the base of each tree. Judging from the results obtained in cage 9 during 1926-27, however, it may be concluded that if the beetles were able to penetrate these masses of needles they would find proper protection for winter survival.



FIGURE 9.—Oak "mottes" found along the streams in the cedar and nut-pine forest zones

The results from cage 10, which contained materials common to the cultivated and sod areas of the Sonoran Zone, showed that there was only one season out of the six in which any beetles survived. Of the 14,500 beetles used in this experiment, 4 lived through the winter of 1923-24. Where oak leaves and pine needles, material which is foreign to the zone, were used as hibernation shelter (cages 11 and 12), 3.6 per cent of the beetles survived. Where protection from the wind was afforded, which allowed for an even distribution of snow and decreased sand coverage, as in cage 11, the percentage of beetles that overwintered averaged 5.59. In cage 12, which contained the same material but which was exposed to the wind, only 0.27 per cent passed the winter during the three years the experiment was in progress.

These facts show that sheltering material found naturally in the open areas of this zone does not provide the beetle with proper coverage for successful winter survival. The greater survival in the cages containing oak leaves and pine needles explains why beetles can hibernate in oak "mottes" (fig. 9) found along streams and

<sup>4</sup> Graf, J. E. Unpublished data.

canyons. The oak mottes represent an outcropping of the Transition Zone at the upper edge of the Upper Sonoran Zone due to topography, exposure, temperature, and soil moisture in that particular vicinity.

#### MISCELLANEOUS FACTORS AFFECTING MORTALITY OF BEETLES

##### DUST STORMS

In the Estancia Valley dust storms occur frequently in the spring and occasionally in other seasons. These storms may last for a few hours or for several days, and the quantity of dust carried at times is enormous. It has been found that the quantity of dust deposited is in direct proportion to the quantity and size of any obstruction in its path and the efficiency of this obstruction as a windbreak. Russian thistle (*Salsola pestifer*) is the most abundant and widely distributed weed in the Estancia Valley. When mature it breaks off at the surface of the ground and rolls before the wind. These weeds finally lodge and pile up along fences (fig. 10), where they

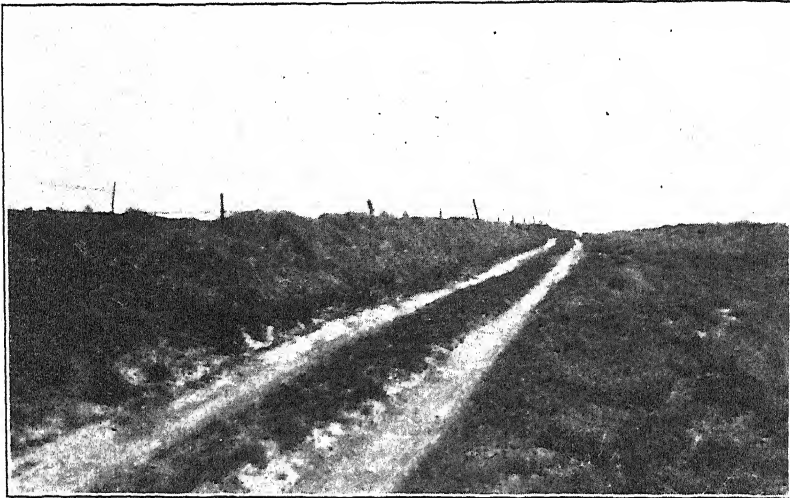


FIGURE 10.—Russian thistles (*Salsola pestifer*) piled up along a fence by the wind

break the force of the wind so that it deposits a large quantity of sand and dust on the leeward side of the fence, until the fence is finally covered. This accumulation alone prevents the successful hibernation of the bean beetle on the alluvial slopes and valley floor.

##### CHARACTER OF HIBERNATION MATERIAL

The character of the hibernation material is an important factor in the successful overwintering of the insect. A suitable material is one that permits beetles to enter and emerge readily, and at the same time protects them both from low temperature and from rapid changes of temperature. It must also be able to retain moisture so that too rapid desiccation of the beetles will not occur. A mixture of oak leaves and pine needles, such as is often found in well-protected places, is the most favorable material. This finding confirms the

results of Howard and English<sup>5</sup> and Thomas<sup>6</sup> obtained in studies of the beetle in the Southern States. Compact material, such as juniper and piñon needles, is unsuitable because the beetles are unable to enter it. Weeds are the most unsatisfactory material because they permit rapid changes in temperature and moisture.

#### SNOWFALL

A protection of snow during periods of subzero weather is essential for successful hibernation. The following example will illustrate this point. On December 19, 1927, a thermograph measuring air temperature registered  $-20^{\circ}$  F., as compared with  $21^{\circ}$  (fig. 12, D) registered by a distance thermograph that was recording the temperature of the hibernation material of oak leaves and pine needles under 8 inches of snow in cage 7 near by. Again on February 10, 1929, the air temperature was  $-27^{\circ}$  at Estancia, as compared with a temperature of  $25^{\circ}$  under a 6-inch blanket of snow in the hibernation material of oak leaves and pine needles in cage 11 near by. (Fig. 12, A.) At the same time the temperature in the hibernation material in the adjacent cage 10, where Russian thistles were used, was  $-1^{\circ}$ . (Fig. 12, B.) Weeds hold the blanket of snow off the ground and permit lowering of temperature. The water content of 1 inch of snow may vary from 0.03 to over 0.10 inch,<sup>7</sup> according to temperature, altitude, etc., and this variation may affect its insulating properties.

#### DRAINAGE

A comparison of the results obtained from cages 5 and 8 shows that drainage is a factor of importance in the successful hibernation of the beetle. Both cages were located on the northern exposure under similar climatic conditions and with the same type of hibernation material. The former cage had good drainage, while the latter stood on ground that was constantly wet by seepage. During the seasons of 1925-26 and 1926-27 the percentage survival in cage 5 was 22.24 and 28.29, respectively, while in cage 8 it was only 1.83 and 10.32.

#### TIME OF KILLING FROST

The time at which the first killing frost occurs is an important factor in winter survival. From 1923 to 1928 the time of the first killing frost ranged from September 14 to October 22, with September 23 as the average. The growing season in the Estancia Valley is comparatively short, and the reproduction period of the Mexican bean beetle is still shorter, for the beetles do not emerge from hibernation until stimulated by the summer rains. Counts of overwintered beetles in the same field in the foothills for the six summers showed that the largest population of old beetles was present from July 8 to 20, with July 14 as the average date. The developmental period from egg to adult ranges from 40 to 45 days during the summer. When the killing frost occurs early and destroys the bean, which is the only host plant in the valley, a large percentage of the newly

<sup>5</sup> HOWARD, N. F., and ENGLISH, L. L. STUDIES OF THE MEXICAN BEAN BEETLE IN THE SOUTHEAST. U. S. Dept. Agr. Bul. 1243, 51 p., illus. 1924.

<sup>6</sup> THOMAS, F. L. LIFE HISTORY AND CONTROL OF THE MEXICAN BEAN BEETLE. Ala. Agr. Expt. Sta. Bul. 221, 99 p., illus. 1924.

<sup>7</sup> Data from correspondence with C. F. Linner, U. S. Weather Bureau, Santa Fe, N. Mex.

emerged beetles are forced into hibernation in a weakened condition, and the hibernation period is lengthened. On the other hand, a late killing frost prolongs the feeding period and shortens the hibernation period. It is therefore evident that a variation of 38 days in the occurrence of the killing frost in autumn is indirectly a factor in the successful hibernation of the insect.

#### PROXIMITY OF HIBERNATION QUARTERS AND SEASONAL RAINFALL

In the West the Mexican bean beetle is not a major pest in any bean-growing district which does not have suitable hibernation quarters comparatively close to the bean area (within 50 to 70 miles) and which does not have a seasonal rainfall of at least 5 inches rather evenly distributed over the hibernation period. Furthermore, this beetle has never been reported as a serious pest where a well-defined dry hibernation season occurs, especially when the dry season is coincident with high temperatures. In the dry-farmed area in eastern Colorado, for example, the beetle is not known to occur, and in Union County, N. Mex., it has never been a pest of economic importance.

#### HIBERNATION IN RELATION TO ALTITUDE

Considered independently of all other factors, and within certain limits, there seems to be no close relation between altitude and successful hibernation of the bean beetle, as is shown by the results for cages 4 and 7, located in the ponderosa pine zone. (Table 3.) In cage 4 a small quantity of juniper needles was used with oak leaves instead of pine needles as was the case in cages 7 and 11.

TABLE 3.—*Effect of altitude on hibernation of Mexican bean beetles*

Cage No.	Elevation	Beetles surviving					
		1923-24	1924-25	1925-26	1926-27	1927-28	1928-29
	<i>Feet</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
4	8,400	4.15	0.45	2.40	16.53	0.30	6.88
7	7,000	23.50	7.26	36.24	16.22	2.30	3.58
11	6,100			21.80	2.06	.92	1.12

The data for 1923-24, 1924-25, 1925-26, and 1927-28 show that a larger percentage of the insects survived at the lower elevation than at the higher. The figures for 1926-27 and 1928-29, on the other hand, show a greater survival at the higher elevation. In the case of cage 11, at a still lower elevation, the survival was smaller for these two seasons, but larger in 1925-26 and 1927-28 than in cage 4 at the highest elevation.

These conflicting data indicate that elevation alone has no real significance within the zone of successful hibernation. It is known that the altitude of the ponderosa pine zone ranges from 6,850 to 8,400 feet on the eastern exposure and from 8,000 to 9,000 feet on the western exposure, with a great variation on the northern and southern exposures in the Manzano Mountain Range. Pearson<sup>8</sup> has found that temperature and moisture, not elevation, are the limiting factors in the distribution of trees in the Southwest.

<sup>8</sup> PEARSON, G. A. FOREST TYPES IN THE SOUTHWEST AS DETERMINED BY CLIMATE AND SOIL. U. S. Dept. Agr. Tech. Bul. 247, 144 p., illus. 1931.



# RELATION OF EXPOSURE TO SURVIVAL OF BEETLES

The results of six seasons' work, as shown in Table 4, justify the conclusion that exposure, at least so far as locations on eastern, south-eastern, and northern slopes are concerned, affects the survival of beetles under mountainous conditions in New Mexico. The important factor in this connection seems to be the topography of the locality as it governs the intensity or degree of shade. Where the hills are low and the slope is comparatively short, as is the case with the situation of cages 5 and 7, exposure considered apart from other factors seems to have no real significance within the zone of hibernation, but it has an important bearing upon beetle mortality in hibernation during certain seasons. The locations of cages 5 and 7 were similar as to topography, drainage, elevation, flora, soil, and hibernation material, and their close proximity permitted precipitation stimulus to affect the beetles in the two cages similarly.<sup>9</sup> The only important difference was in exposure.

TABLE 4.—*Effect of elevation and exposure on hibernation of Mexican bean beetles*

Cage No.	Elevation	Exposure	Beetles surviving					
			1923-24	1924-25	1925-26	1926-27	1927-28	1928-29
	<i>Feet</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
2-----	8,400	North-----		0	0	1.12	0	0
4-----	8,400	Southeast-----	4.15	0.45	2.40	16.53	0.30	6.88
5-----	7,050	North-----		10.03	22.24	28.20	.75	2.95
7-----	7,000	East-----	23.50	7.26	36.24	16.22	2.30	3.58
8-----	6,975	North-----			1.83	10.32	1.80	.04

There was no cage in operation on the northern slope during the season of 1923-24. During the winters of 1925-26, 1927-28, and 1928-29 more insects survived in cage 7 on the eastern slope than in cage 5 on the northern. In 1924-25 and 1926-27, however, more beetles hibernated successfully on the northern slope. The average survival for the five winters shows no significant difference for the two exposures, being 12.24 per cent for the northern and 11.55 per cent for the eastern. When each year is considered separately, however, and such other factors as sunlight, evaporation, temperature, and soil moisture are taken into consideration, it is evident that exposure has a direct influence on the beetle during its dormant period. It will be shown later that in 1924-25 and 1926-27, when more beetles survived on the northern than on the eastern slope, the winters were followed by mild, dry springs, whereas in 1925-26, 1927-28, and 1928-29, when more beetles hibernated on the eastern slope, the springs were cold and wet. A study of beetle hibernation on any one exposure will therefore not give a true index of winter mortality from season to season, as is indicated in Figure 11.

Where the slope is steep and the distance from the cage to the top of the slope is comparatively great, as in cages 2 and 4, exposure considered apart from other factors seems to have a real significance. These two cages were located at the same elevation less than 300 yards

<sup>9</sup> DOUGLASS, J. R. PRECIPITATION AS A FACTOR IN THE EMERGENCE OF *EPILACHNA CORRUPTA* FROM HIBERNATION. Jour. Econ. Ent. 21: 203-213, illus. 1928.

apart on an eastern spur of Bosque Mountain. Cage 2 was near the lower edge of the fir-spruce association and cage 4 at the upper edge of the ponderosa pine zone, as in this vicinity the Canadian and Transition Zones dovetail into each other on account of the topography and slope exposure. The general air temperature and the precipitation occurring over the two cages were obviously very nearly the same. Cage 4, located on the southeastern slope, was exposed to the sun's rays longer than cage 2 on the northern slope; consequently, the evaporation was greater and the temperature of the duff higher on the southeastern slope. Because of the lower temperature the snow lasts longer on the northern slope, which makes it appear to have more

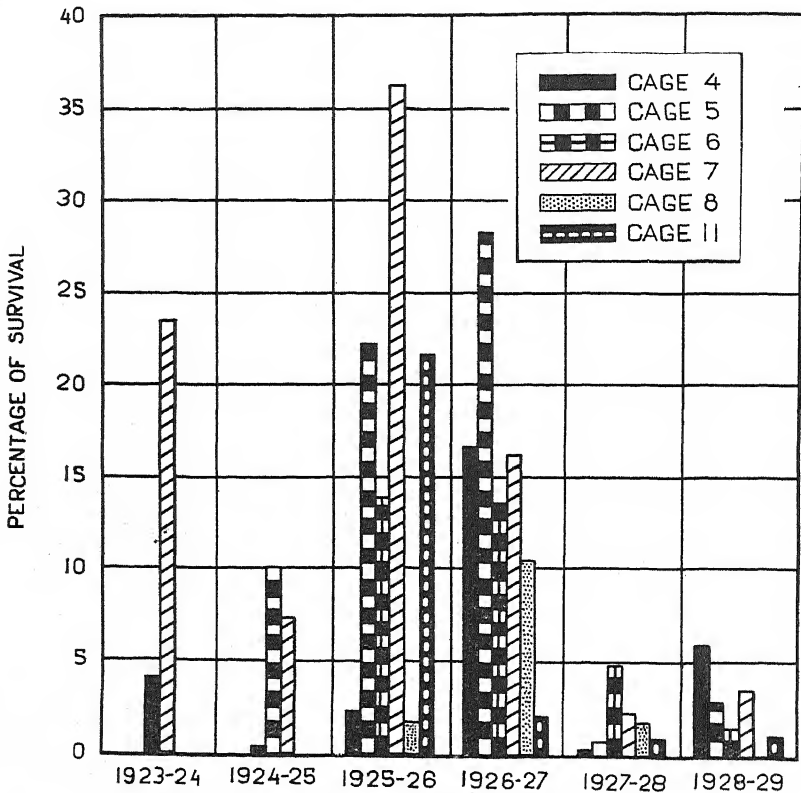


FIGURE 11.—Percentage of beetles surviving hibernation in different cages

moisture than the warmer slopes of the mountain. During the last five seasons of the experimental work 14,000 beetles were introduced into cage 2 and 8,500 into cage 4. In cage 2, 28 beetles survived and in cage 4, 468. (Table 2.) From these results it is apparent that at the same elevation in cages 300 yards apart more beetles may survive in one exposure than in the other.

The important part played by slope exposure and topography in winter mortality may be shown in still a different way by comparing the data given in Tables 1 and 2 for cage 8 with those for the neighboring cage 7. During the four seasons beginning with 1925-26,

the average survival of the 40,000 beetles in cage 7 on the eastern slope was 12.08 per cent as compared with 3.16 per cent for the 25,000 beetles in cage 8 on the northern slope. These data indicate that within the lower part of the ponderosa pine zone, the beetle's natural hibernation quarters in the West, there are certain localized areas that are not suitable for successful overwintering on account of the topography and slope exposure.

HIBERNATION IN RELATION TO TEMPERATURE

A study of the monthly minimum temperatures at Estancia and Tajique (Table 5) for the hibernation seasons under discussion, in connection with Table 2, shows that the lowest temperatures occurred in years that were favorable to hibernation. During the winter of 1924-25, when  $-17^{\circ}$  F. was recorded for Tajique, 10.03 per cent of the beetles introduced into cage 5 overwintered. Again during the season of 1925-26,  $-24^{\circ}$  was recorded at Estancia and 21.8 per cent of the beetles survived in cage 11. The lowest temperature ( $-26^{\circ}$ ) occurred at Estancia and yet beetles survived this low air temperature, while in the fir-spruce association on Bosque Mountain (Rea's ranch), where the lowest temperature recorded in five years was  $-15^{\circ}$ , there was 100 per cent mortality in the cages in four out of five years of the investigational period.

TABLE 5.—*Monthly minimum temperatures at Estancia and Tajique, N. Mex., and the lowest monthly temperatures recorded at Rea's ranch, 1923-24 to 1928-29*

Station	Season	October	November	December	January	February	March	April	May	June
		$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.	$^{\circ}$ F.
Estancia	1923-24	23	1	-16	-2	-2	8	15	27	33
	1924-25	17	2	-26	-11	8	3	21	27	30
	1925-26	11	4	-5	-24	5	0	20	23	39
	1926-27	18	12	-16	-1	12	10	19	28	31
	1927-28	17	4	-10	-5	-5	11	1	20	35
	1928-29	21	13	-1	-4	-22	8	15	17	36
	1923-24	12	-2	-2	2	-1	3	13	24	31
	1924-25	20	10	-17	-4	14	10	22	32	33
Tajique	1925-26	24	4	0	-4	10	0	24	23	38
	1926-27	15	15	-10	12	13	5	20	26	36
	1927-28	20	13	-13	-4	-3	8	-4	30	34
	1928-29	19	10	-1	-2	-13	1	13	13	34
Estancia	Lowest	11	1	-26	-24	-22	0	1	17	30
Tajique	do.	12	-2	-17	-4	-13	0	-4	13	31
Rea's ranch <sup>a</sup>	do.	14	0	-6	-8	-15	-1	10	12	23

<sup>a</sup> Temperatures for period 1913 to 1917; no weather station in operation during the investigational period.

The weekly thermograph records showing the temperatures of the air and of the hibernation material, as presented in Figure 12, illustrate why beetles under the cover of hibernation materials are able to survive during periods when the air temperatures above the materials are extremely low. During periods of subzero weather the ground is usually covered with snow, the protection of which is discussed elsewhere in this paper. The records for April 9, 1928, and May 2, 1929, show that the high mortality occurring during the seasons of 1927-28 and 1928-29 was not caused by low air temperature on these dates, as the temperature of the hibernation material did not go below  $30^{\circ}$  F.

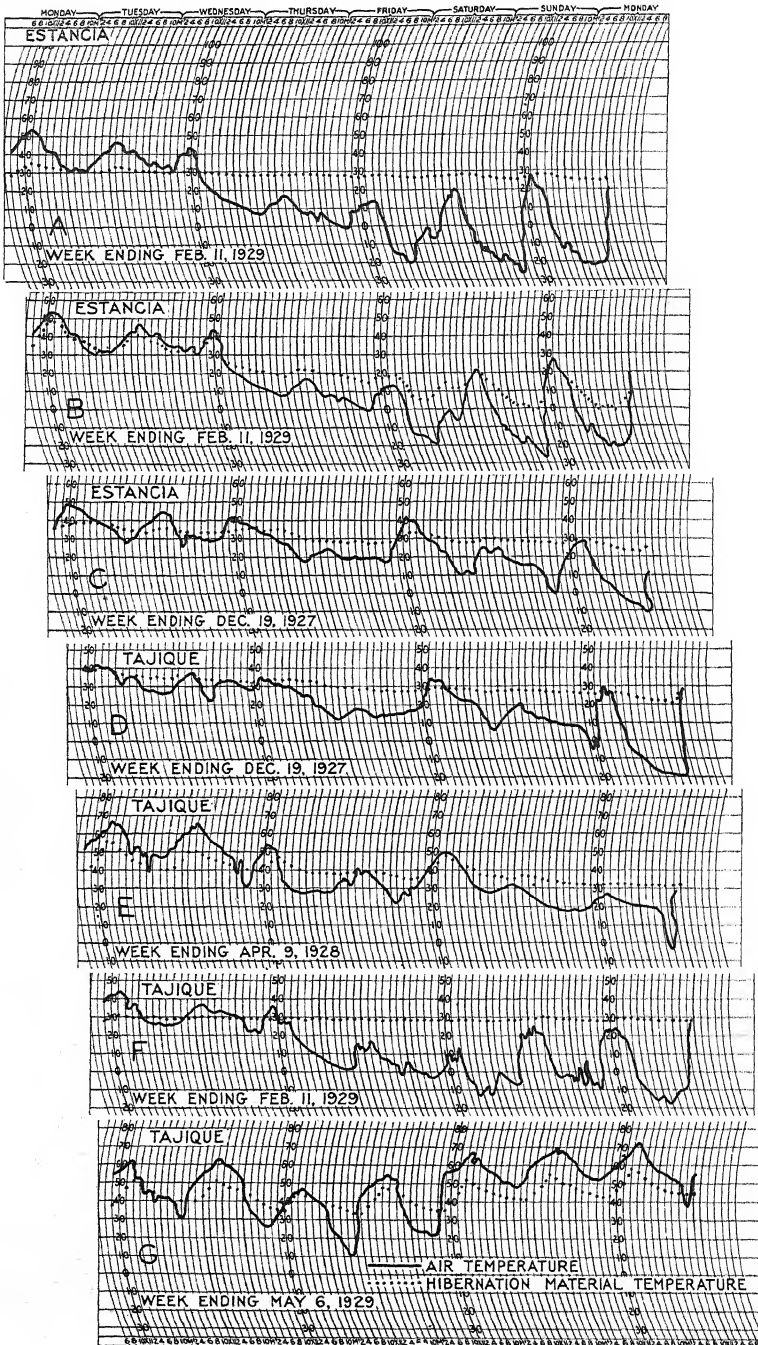


FIGURE 12.—Weekly thermograph records showing the temperatures of the air and of the hibernation material. A is a record taken in cage 11, in which the hibernation material consisted of oak leaves and pine needles, whereas B is a record for the same period in adjacent cage 10, where Russian thistles were used. In all other cases the hibernation material consisted of oak leaves and pine needles.

In Table 6 and Figure 13 it is found that the mean minimum temperatures for November, December, and January are lower at Estancia than at Rea's ranch on Bosque Mountain or in the foothills (Tajique), while from February to June, inclusive, these temperatures are lower

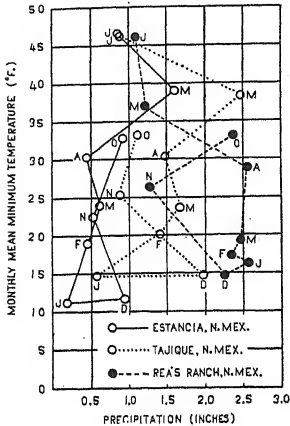


FIGURE 13.—Climograph showing mean monthly minimum temperatures at Estancia, Tajique, and Rea's ranch, N. Mex.

on the mountain than in the foothills or valley. Of the beetles introduced over a period of five years into cages located on the mountain (Nos. 1, 2, and 3), none survived except during the season of 1926-27, when the monthly mean minimum temperature from January to April, inclusive, at Estancia and Tajique rose 19.3° and 15.5° F., respectively, above the average mean for this period. This mild season, which prevailed generally over the area, permitted successful hibernation even at the higher elevations. This is best illustrated by the results obtained in cage 4 at 8,400 feet elevation, where the average survival for the mild season of 1926-27 was 16.53 per cent as compared with 3.57 per cent for the other five seasons. In cage 8 also during the mild season 10.32 per cent of the beetles survived as compared with an average of 1.38 per cent for the season

preceding and the two seasons following the mild winter. It will therefore be seen that the temperatures of the latter part of the hibernating period have the greatest influence on survival.

TABLE 6.—Monthly mean minimum temperatures at Estancia and Tajique, N. Mex., and the mean of the monthly mean minimum temperatures there and at Rea's ranch, 1923-24 to 1928-29

Station	Season	October	November	December	January	February	March	April	May	June
		°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Estancia	1923-24	32.8	23.3	15.8	12.1	18.4	21.7	29.2	39.4	47.1
	1924-25	30.3	19.4	7.2	8.8	20.6	22.4	30.2	39.8	46.2
	1925-26	33.3	17.1	9.2	-2.1	18.3	22.1	30.8	36.0	45.8
	1926-27	33.8	24.0	9.8	21.8	25.5	24.2	32.1	37.5	45.6
	1927-28	28.2	25.6	11.3	11.3	19.6	26.0	30.1	40.7	46.9
	1928-29	37.5	25.0	15.6	15.1	11.9	26.4	29.4	40.4	47.2
Tajique	1923-24	31.1	22.6	16.6	13.0	19.6	20.3	27.8	38.0	46.9
	1924-25	30.8	24.4	13.2	11.1	23.8	26.8	32.6	41.6	47.1
	1925-26	35.3	23.4	16.6	10.9	23.0	22.0	33.0	26.6	44.2
	1926-27	35.3	27.2	14.2	21.4	26.3	23.9	32.8	37.4	43.2
	1927-28	31.1	30.8	12.9	19.1	16.7	25.4	28.6	38.8	50.4
	1928-29	35.3	22.5	15.1	13.4	11.2	23.1	27.8	37.9	46.0
Estancia	Mean	32.7	22.4	11.5	11.2	19.0	24.0	30.3	39.0	46.5
Tajique	do	33.2	25.2	14.8	14.8	20.1	23.6	30.4	38.4	46.3
Rea's ranch	do	33.0	26.4	14.8	16.3	17.3	19.3	28.9	37.0	46.0

• Data recorded between 1913 and 1917.

A further study of Table 6 shows that for Estancia during the hibernation season of 1925-26 the sum of the monthly mean minimum temperatures for November to March, inclusive, decreased 23.5° F. from the sum of the means for the same period. During this season

21.80 per cent of the beetles introduced into cage 11 survived as compared with an average survival of 1.54 per cent for the three succeeding years. (Table 2.) These conflicting data show that other important factors besides air temperature are exerting an influence upon the winter mortality of bean beetles.

HIBERNATION IN RELATION TO TEMPERATURE AND PRECIPITATION

The moisture requirements of the beetles vary directly with the temperature, as is indicated in Table 7, which gives the relation between beetle survival and the average seasonal temperature and precipitation at stations in various parts of the range of the beetle in this country.

TABLE 7.—Average seasonal temperature, precipitation, and beetle survival at several stations

Station	Period	Hibernation period	Temperature	Precipitation	Beetle survival
			°F.	Inches	Per cent
Tajique, N. Mex.	1923 to 1929	October to June.	40.35	12.47	<sup>a</sup> 12.60
Rea's ranch, N. Mex.	1924 to 1929	do.	35.94	18.08	.11
Columbus, Ohio.	1925 to 1929	October to May.	42.71	23.76	<sup>b</sup> 1.43
Amesville, Ohio.	do.	do.	43.94	<sup>c</sup> 24.64	<sup>b</sup> 2.38
Birmingham, Ala.	1920 to 1928	November to April.	52.83	30.36	<sup>b</sup> 15.41
Clemson College, S. C.	1924 to 1929	do.	50.12	27.43	<sup>d</sup> 15.76

<sup>a</sup> For cages 5 and 7.  
<sup>b</sup> From unpublished data by N. F. Howard, Bureau of Entomology.  
<sup>c</sup> For Athens, Ohio.  
<sup>d</sup> Calculated from data given by EDDY, C. O., and CLARKE, W. H. THE MEXICAN BEAN BEETLE, 1927-1928. S. C. Agr. Expt. Sta. Bul. 258, 42 p., illus. 1929.

The climographs for these stations, presented in Figure 14, show the average monthly temperature and precipitation during the hibernation period under discussion.

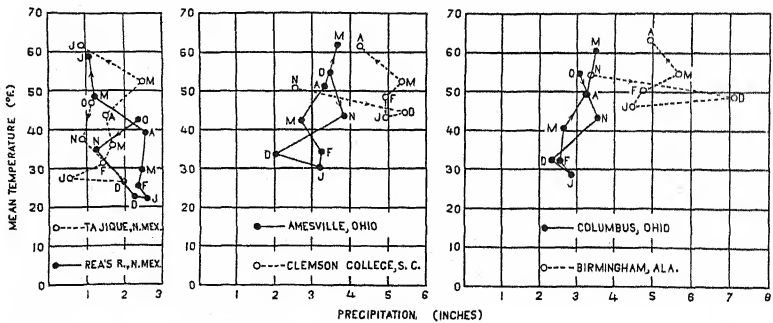


FIGURE 14.—Climograph showing mean monthly temperatures and precipitation in different parts of the range of the Mexican bean beetle in the United States

The average temperature at Tajique, N. Mex., is 2.36° F. lower than at Columbus, Ohio. From the standpoint of temperature, therefore, Columbus is more favorable for winter survival, but exces-

sive moisture combined with low temperature makes it unfavorable for successful hibernation. Tajique, on the other hand, with 11.29 inches less moisture, is favorable for survival. There is little difference in the percentage survival at Birmingham, Ala., and Tajique, N. Mex.; yet the latter station is 12.48° colder and has 17.89 inches less precipitation.

A few beetles have lived 3 days submerged in running water, although the mortality was high on the first and second days. Beetles in perforated salve boxes containing oak leaves placed under a dripping faucet lived from 1 to 12 days, but heavy mortality occurred between the first and fourth days.

## HIBERNATION AS AFFECTED BY CLIMATIC FLUCTUATIONS

### SEASONAL FLUCTUATIONS

The data in Table 8 and the climographs for Estancia and Tajique (fig. 15) show large variations, from season to season, in temperature and precipitation for corresponding months during the hibernation

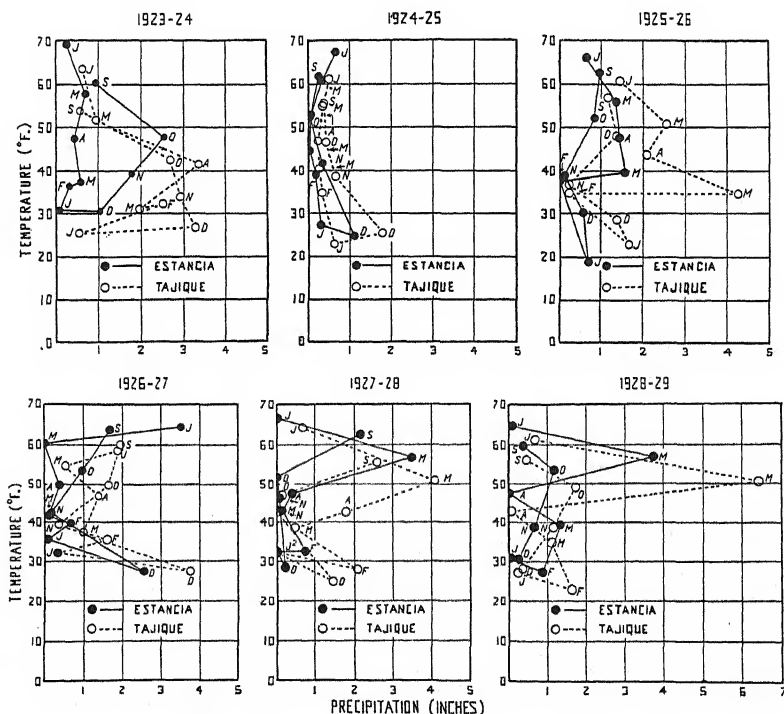


FIGURE 15.—Climographs for Estancia and Tajique, N. Mex.

period. This fluctuation in climatic conditions seems to be the most important factor affecting hibernation in the same cage. A single departure from the normal is not always important, but the combination or the sequence of such departures is important.

TABLE 8.—Climatic data for Estancia and Tajique, N. Mex., 1923-24 to 1928-29

MONTHLY MEAN TEMPERATURES												
Station	Hibernation season	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
		° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.
Estancia	1923-24	60.3	47.8	39.3	30.2	30.7	36.2	37.1	47.5	57.8	69.1	69.7
	1924-25	61.8	52.1	41.6	24.8	27.2	39.0	44.7	52.9	60.7	67.1	72.6
	1925-26	62.3	52.0	38.9	30.1	18.8	37.6	39.5	47.7	55.7	66.0	67.4
	1926-27	63.9	53.6	42.1	27.3	35.6	39.8	41.3	50.0	60.2	64.5	70.4
	1927-28	62.5	51.8	45.6	28.2	32.5	32.4	42.9	47.1	56.8	66.6	68.4
	1928-29	59.7	53.2	38.4	30.3	30.5	27.1	39.2	47.5	56.8	64.7	68.5
	1923-24	53.6	42.4	33.8	26.8	25.2	32.0	31.0	41.4	51.8	63.6	62.4
Tajique	1924-25	55.4	46.2	38.7	25.4	22.8	34.2	40.2	46.6	54.8	61.0	64.8
	1925-26	56.7	48.0	36.5	28.5	22.8	34.6	34.2	43.6	50.8	60.6	61.8
	1926-27	60.0	49.5	39.1	27.1	33.5	35.2	37.2	46.4	54.6	58.6	64.2
	1927-28	55.6	46.8	42.4	24.9	32.4	27.9	38.1	42.2	50.9	64.4	64.3
	1928-29	56.0	49.0	34.3	28.0	26.9	22.9	34.8	42.8	50.7	61.0	62.9

MONTHLY PRECIPITATION												
		Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches
Estancia	1923-24	0.91	2.51	1.78	1.02	0.07	0.30	0.59	0.42	0.70	0.26	1.88
	1924-25	.24	.05	.32	1.09	.30	.20	.04	.08	.33	.65	2.03
	1925-26	.99	.82	.13	.58	.70	.02	1.54	1.43	1.37	.63	2.20
	1926-27	1.65	.97	.15	2.54	.06	.64	.05	.37	(a)	3.50	2.30
	1927-28	2.13	(a)	.02	.20	0	.73	.13	.39	3.47	(a)	.74
	1928-29	.36	1.14	.67	.23	.03	.88	1.31	(a)	3.75	.07	5.47
	1923-24	.57	2.66	2.90	3.23	.53	2.49	1.98	3.35	.92	.62	5.41
Tajique	1924-25	.40	.43	.65	1.77	.61	.44	.26	.24	.38	.51	2.62
	1925-26	1.14	1.37	.23	1.39	1.69	.23	4.24	2.09	2.55	1.47	2.14
	1926-27	1.95	1.63	.35	3.77	.32	1.60	.99	1.38	.51	1.86	1.70
	1927-28	2.57	.11	0	1.43	0	2.06	.44	1.80	4.08	.05	2.98
	1928-29	.47	1.72	1.13	.31	.21	1.63	2.09	.05	6.42	.68	6.50

## TOTAL MONTHLY SNOWFALL (UNMELTED)

		Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches	Inches		
Estancia	1923-24	4.5	4.5	13.5	1.0	3.9	10.2	2.2	0	0	-----	-----
	1924-25	0	0	13.2	7.2	1.5	0	(a)	0	0	-----	-----
	1925-26	(a)	.5	5.0	10.0	.2	10.5	0	0	0	-----	-----
	1926-27	2.0	0	9.3	(a)	1.0	.2	1.0	0	0	-----	-----
	1927-28	0	0	4.8	0	12.0	.5	4.5	(a)	0	-----	-----
	1928-29	0	3.0	1.0	(a)	18.0	10.0	0	1.0	0	-----	-----
	1923-24	25.5	21.0	46.5	7.0	29.0	19.7	20.7	.5	0	-----	-----
Tajique	1924-25	.5	0	27.2	11.3	3.3	5	0	0	0	-----	-----
	1925-26	(a)	3.0	13.5	24.6	2.7	37.8	(a)	(a)	0	-----	-----
	1926-27	5.5	8	39.3	3.0	8.1	8.0	13.0	1.0	0	-----	-----
	1927-28	0	0	21.2	0	31.2	(a)	17.1	19.4	0	-----	-----
	1928-29	.2	9.5	4.8	3.9	26.6	19.0	(a)	2.0	0	-----	-----

<sup>a</sup> Trace.

The departures from the normal of temperature and precipitation at Tajique are shown in Table 9.

During the season of 1923-24 the fall was relatively cold and wet, and it was followed by a cold, damp winter with the heaviest snowfall on record. May was nearly normal in temperature, but far below normal in precipitation. June was hot and dry, so that emergence from hibernation was delayed until the July rains. As a whole the hibernation season was the coldest and wettest recorded. However, the winter was favorable for hibernation because the beetles were well protected by a heavy blanket of snow during the cold months, and a mild dry spring followed which was unfavorable for the parasitic fungus *Beauveria globulifera*. In cage 7, on an eastern slope, 23.5 per cent of the beetles survived. It is thus apparent that certain determining factors (in the present instance temperature and pre-



cipitation as they affect the development of parasitic fungi) may be so influenced by other determining factors, such as form of precipitation (snow instead of rainfall) or the time of occurrence of the precipitation, as to counteract the unfavorable effects of the first factors.

TABLE 9.—*Departures from the normal of temperature and precipitation, at Tajique, N. Mex., 1923-24 to 1928-29*

[7,100 feet elevation]						
TEMPERATURE						
Month	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29
	° F.	° F.	° F.	° F.	° F.	° F.
October.....	-4.2	-0.4	+1.4	+2.9	+0.2	+2.4
November.....	-3.3	+1.6	-6	+2.0	+5.3	-2.9
December.....	-1.8	-3.2	-1	-1.5	-3.7	-6
January.....	-3.1	-5.5	-5.5	+5.2	+4.1	-1.5
February.....	+1.0	+3.2	+3.6	+4.2	-3.1	-8.1
March.....	-4.8	+4.4	-1.6	+1.4	+2.3	-1.0
April.....	-1.8	+3.4	+4	+3.2	+1.0	+6
May.....	-4	+2.6	-1.4	+2.4	-1.3	-1.5
June.....	+2.7	+1	-3	-2.3	+3.5	-1
Seasonal average.....	-1.8	+7	-5	+2.0	+9	-1.4
PRECIPITATION						
	Inches	Inches	Inches	Inches	Inches	Inches
October.....	+1.34	-0.87	+0.05	+0.31	-1.21	+0.40
November.....	+2.11	-14	-56	-44	-79	+34
December.....	+1.75	+29	-09	+2.29	-05	-1.17
January.....	-39	-31	+77	-60	-92	-71
February.....	+1.24	-81	-1.02	+35	+81	+38
March.....	+55	-1.17	+2.81	-44	-99	+66
April.....	+1.85	-1.26	+59	-12	+30	-1.45
May.....	-1.15	-1.69	+48	-1.56	+2.01	+4.35
June.....	-53	-64	+32	+71	-1.10	-47
Seasonal average.....	+76	-73	+37	+05	-21	+26

The season of 1924-25 was mild and dry, except for December and January, which were cold, with a good protection of snow. The precipitation was well distributed throughout the hibernation period, but the deficiency was too great for favorable hibernation. On the eastern slope (cage 7) 7.26 per cent and on the northern exposure (cage 5) 10.03 per cent of the beetles hibernated, indicating that the deficiency of moisture in the hibernation material was greater on the eastern than on the northern exposure.

During the season of 1925-26 the fall temperature was moderate with a slight deficiency in moisture. The winter was variable, with a good blanket of snow during the cold months and an excess of moisture in March. The spring was slightly above normal in precipitation, which stimulated emergence in June. The season as a whole was nearly normal and favorable for overwintering. In the cage on the eastern slope 36.24 per cent and in that on the northern slope 22.24 per cent of the insects overwintered, indicating that the northern exposure received a slight excess of moisture above the optimum.

The season of 1926-27 was the warmest on record, with a slight excess of precipitation. December was the wettest month and May the driest. The spring was hot and dry, which conditions increased

mortality on the eastern slopes and decreased the death rate on the northern. On the eastern exposure 16.22 per cent of the beetles hibernated as compared with 28.29 per cent on the northern exposure. The excess rainfall in June stimulated emergence and thus decreased the length of the hibernation period.

The season of 1927-28 was comparatively warm, with two winter months below normal in temperature and six of the nine months deficient in precipitation. January was warm, clear, and exceedingly dry, the driest on record. May was below normal in temperature, and above normal in precipitation. Snow fell during December, February, April, and May; the amount falling in May was the largest recorded for that month. (Table 8.) June was hot and dry, and emergence of the beetles was delayed until the July rains. The season as a whole was slightly above normal in temperature, with a small decrease in precipitation. On the eastern exposure 2.30 per cent of the beetles survived the winter, and on the northern exposure 0.75 per cent.

For the winter of 1928-29 October and April were above normal in temperature, October being one of the warmest on record. The other months were below normal in temperature, February being the coldest—in fact, it was the coldest February recorded. March was cold and wet, with a heavy, wet snowfall on the 27th and 28th. Five of the nine months had an excess of precipitation, that for May being the greatest on record. June was warm and dry, with a slight precipitation which occurred at the close of the month. Emergence from hibernation was delayed until the July rains. The season as a whole was cold with a slight excess of precipitation. It was unfavorable for hibernation. In the cage on the northern slope 2.95 per cent of the beetles survived as compared with 3.58 per cent on the eastern slope.

Figure 11 shows that the seasons of 1923-24, 1925-26, and 1926-27 were favorable for winter survival, that 1924-25 was fair in this respect, and that 1927-28 and 1928-29 were the least favorable. The seasonal average departures from normal temperature and precipitation, as given in Table 9, show no correlation with winter survival. They do, however, fall within the seasonal variations in survival for the good winters. The only significant relationship is shown between the comparatively uniform seasons of 1923-24 and 1924-25, the cool, damp winter being more favorable for survival than the warm, dry season.

#### EFFECT OF A COVERING OF SNOW IN RELATION TO TEMPERATURE FLUCTUATIONS

Tables 8 and 9 show a great variation in monthly temperatures from season to season at Tajique. For example, the temperatures in January, 1926 and 1927, showed a variation of 10.7° F. The snowfall for these months (Table 8) was 24.6 and 3.0 inches, respectively. In 1925-26, 36.24 per cent of the beetles survived in cage 7, as compared with 16.22 per cent in the same cage in 1926-27. March in 1924 and 1925 varied 9.2° in temperature, with 19.7 and 0.5 inches, respectively, of snowfall. In cage 7, 23.54 per cent of the beetles survived in 1923-24 as compared with 7.26 per cent in 1924-25.

The mean temperature at Tajique for January, 1925, and for January, 1926, was 22.8° F., with 11.3 and 24.6 inches of snowfall,

and in 1924-25 and 1925-26, 7.26 and 36.24 per cent of the beetles, respectively, survived in cage 7. Of the beetles in cage 11 (at Estancia), 21.80 per cent survived during the winter of 1925-26, when the monthly mean temperature decreased to 18.8° for January with a 10-inch snowfall. The mean minimum temperature for this month was -2.1°. (Table 6.)

These data show that during the coldest months of the year the beetles are usually well protected by a blanket of snow and that when there is sufficient covering of snow very few are killed. The lowest temperature recorded in the last three seasons by distant thermographs measuring temperature of natural hibernation material of oak leaves and pine needles was 16° F. In the Southwest the coldest periods follow snowfalls and are accompanied by clear weather.

#### PRECIPITATION FLUCTUATIONS

During the season of 1923-24, October, November, and December had an excess of precipitation of 1.34, 2.11, and 1.75 inches (Table 9), and during the season of 1925-26 March had an excess of 2.81 inches. In 1926-27 December had an excess of 2.29 inches. This excess precipitation during the months mentioned had little effect on mortality, which indicates that these are not the critical months in the hibernation of the beetle. During the season of 1923-24 April had an excess of 1.85 inches of moisture without any great effect on the mortality of beetles in hibernation. In 1927-28 May was apparently the critical month in the hibernation of the insect. The heavy snowfall during April and May (Table 8) was evidently the cause of the high mortality, especially that in May, when precipitation occurred on 14 days. This conclusion is supported by the fact that 4.92 per cent of the beetles survived in cage 6, from which precipitation was excluded after May 10, as compared with 2.30 per cent survival in cage 7 near by.

During the season of 1928-29 May was cool and the precipitation recorded was the greatest, indicating that this was the critical month of the season. In cage 7, 3.58 per cent of the beetles survived as compared with 1.52 per cent in cage 6. The greatest mortality must have occurred before May 10, when natural precipitation was excluded from cage 6. This indicates that heavy precipitation in the form of rain in May, 1929, was not so detrimental as the heavy snowfall in May, 1928. The critical period must therefore have been subsequent to the heavy snowfall on March 27 and 28. Following this period there were 10 days in which the mean temperature averaged 42.7° F. as compared with 45° for the 10-day period subsequent to the heavy snowfall on May 11 and 12, 1928. The weather conditions during these periods were very favorable for the fungus *Beauveria globulifera*, which caused high mortality among the hibernating beetles. Cage examination during the latter part of April, 1929, showed that many had died before that time.

Cage examinations during the period of hibernation showed that very few beetles are killed during complete dormancy in the natural hibernation zone and that the period of heavy mortality occurs with and immediately following the melting of the accumulated snow, when the beetles are becoming semiactive and are absorbing moisture. For example, during the 1925-26 season beetles in cage 8 were in fine

condition until the snow began to melt, and cage examination on April 8 indicated that a large percentage of them would survive. At this time the beetles were becoming semiactive, as five of them were up on the cage, evidently to avoid the excess moisture. After the snow began to melt on the northern slope, where this cage was located, the hibernation material was saturated with moisture for a long time. Later examinations showed that the beetles were being killed by *Beauveria globulifera*, and on May 9 very few live ones were found. Only 1.83 per cent survived. During the warm, dry spring of 1927, 10.32 per cent of the beetles survived in this cage, whereas in the wet springs of 1928 and 1929 only 1.80 and 0.04 per cent survived.

### SUMMARY AND CONCLUSIONS

The hibernation of the Mexican bean beetle in the Upper Sonoran, Transition, and Canadian Life Zones has been studied in relation to the plant associations found therein. In the fir-spruce association of the Canadian Zone only 42 insects survived out of a total of 38,500 placed in hibernation during the five seasons 1923-24 to 1928-29, inclusive. This survival occurred during an abnormally warm winter with nearly normal precipitation. In the ponderosa pine, or Transition, zone, 133,340 beetles were used in six years (1923-24 to 1928-29, inclusive), with a total survival of 13,114, or 9.84 per cent. In the Upper Sonoran Zone 44,500 beetles were employed, with a survival of 1,210 adults, or 2.72 per cent. Under natural conditions hibernation is confined to oak mottes found along canyon streams near the upper edge of this zone. The comparison of these results shows a consistent relationship between the zones and the percentage survival. The results indicate that the ponderosa pine forest zone is the natural hibernation quarters of the beetle in the Southwest and becomes more favorable when oak trees are present in the association.

The accumulation of dust, the character of the hibernation material, and snow coverage during subzero weather have been found to affect mortality in hibernation. Drainage is also an important factor in hibernation, mountainous or hilly country being more favorable for winter survival than a low, flat country when other conditions are similar.

Elevation considered alone seems to have no significance within the zone of successful hibernation. The determining factors at high elevations are temperature, precipitation, and exposure.

In the ponderosa-pine forest zone during winters followed by mild, dry springs, the largest percentage of beetles survived in cages on a northern exposure, whereas during winters followed by cold, wet springs survival was greatest on an eastern exposure. A sunny exposure may make hibernation possible in a location otherwise too cold or damp.

Winter temperature is an important limiting factor in the distribution of the Mexican bean beetle. Unless other factors are favorable, such as a covering of snow and the character of the hibernation material, the temperatures in many sections of the northern part of the United States and in Canada are rather low for successful hibernation.

Precipitation is a major factor in successful hibernation. As the temperature decreases, so does the moisture requirement or toleration

of the insect. The seasonal distribution of precipitation seems to be more important than the quantity. Spring is the critical period in hibernation. Rain is not so detrimental as snow in the spring, especially if the snow is wet and heavy.

The parasitic fungus *Beauveria globulifera* is capable of causing a high death rate among overwintering beetles. The optimum conditions for growth and reproduction of this fungus follow heavy snowfalls or damp, rainy weather in the spring when the mean temperature is between 42° and 45° F.

Very few beetles are killed during complete dormancy; the period of heavy mortality occurs when the beetles become semiactive.



# OLFACTORY RESPONSES OF BLOWFLIES, WITH AND WITHOUT ANTENNAE, IN A WOODEN OLFACTOMETER<sup>1</sup>

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## INTRODUCTION

In 1924 the writer began a series of studies dealing with the tropisms of insects. Shortly after the work was started it became evident that if the fundamentals of attractants and repellents were to be studied, special apparatus for measuring or recording the responses of insects to these substances must be developed. Later the writer (5)<sup>2</sup> devised and perfected such an apparatus and called it an "insect olfactometer." This type of apparatus was suitable only for slowly crawling insects, however, such as the Colorado potato beetle. Since 1926 several other investigators have made and used other types of olfactometers, but most of these have proved unsatisfactory.

In the spring of 1930 the present study was begun at the laboratory of the Bureau of Entomology at Takoma Park, Md. House flies were tested in special wire-screen cages, and it was soon learned that the flies can readily distinguish differences not only between the four classes of substances that induce the four human attributes of taste but also between various sweet liquids. The method used was suitable for determining the food preferences of flies as it let them come in contact with the substances to be tested, but it was not suitable for studying the fundamentals of attractants and repellents. After much work a satisfactory olfactometer was devised. This apparatus is called the wooden olfactometer to distinguish it from the glass olfactometer described by the writer in 1926. All attempts to use a modified form of the olfactometer made and successfully used with the Natal fruit fly (*Ceratitis rubivora* Coq.) by Ripley and Hepburn (7) were unsatisfactory for obtaining positive results quickly.

The original plan was to make a special study of the house fly (*Musca domestica* L.), but as the blue-bottle blowfly (*Calliphora erythrocephala* Meig.),<sup>3</sup> the green-bottle blowfly (*Lucilia sericata* Meig.), and the black blowfly (*Phormia regina* Meig.) reacted more satisfactorily in this type of olfactometer, they were used almost exclusively. In fact, these three species of blowflies proved to be ideal insects for this particular study.

## METHOD OF REARING FLIES

Flies were reared in wire-screen cages (fig. 1), 12 inches square and 18 inches tall. Each cage had a drawer (a) 4 inches deep which was kept half full of moist sand. The flies were fed 10 per cent sugar water in small earthenware dishes (b), 3 inches in diameter and 1.25 inches

<sup>1</sup> Received for publication, Aug. 31, 1932; issued May, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 625.

<sup>3</sup> The blowflies were identified by J. M. Aldrich, of the U.S. National Museum.

deep, and to prevent the insects from drowning in the liquid a disk of perforated cork was allowed to float on the surface of the sugar water. The flies were given tap water periodically in similar containers. To induce oviposition the blowflies were given Hamburg steak (c) in other dishes and the house flies laid eggs on moist bran in the box where their larvae later pupated.

Steak, bearing fly eggs, was put in battery jars (fig. 2, a), 6.5 inches in diameter and 8 inches tall, which contained sand and a supply of meat (b). The jars were then covered with cheesecloth and put in a dark box, where the larvae developed and finally pupated in the sand. When the imagoes began to emerge, the jars were removed

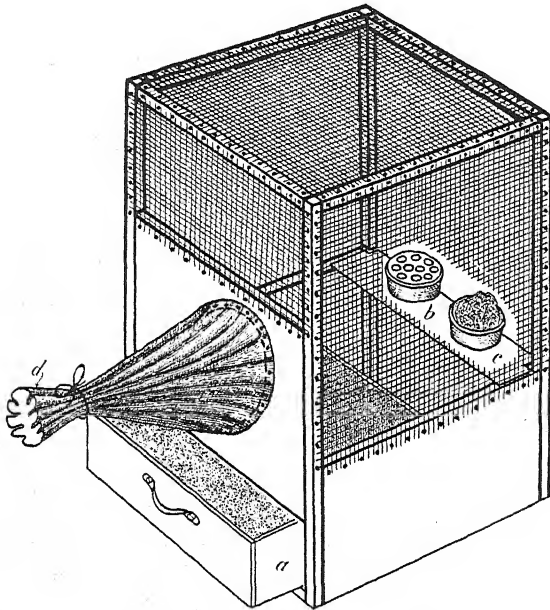


FIGURE 1.—Wire-screen cage in which flies were reared: a, Drawer half full of moist sand; b, small earthenware dish containing 10 per cent sugar water, on the surface of which floats a disk of perforated cork; c, earthenware dish containing Hamburg steak; d, black cloth cylinder used as an entrance and exit

from the dark box to a light place and flytraps (c) were placed over them. If the flies emerged slowly they were fed sugar water on pieces of cotton, suspended from stoppers in the tops of the traps. Periodically the flies were transferred to the cages by inserting the flytraps into cylinders (fig. 1, d) of black cloth, which were fastened around large holes in the cages. By this method large supplies of flies of known age were obtained.

When the weather was sufficiently warm the flies were reared on a south porch adjoining the

writer's laboratory, but during the winter months the cages containing flies were kept on a temporary table built over a radiator by a west window of the laboratory. The battery jars containing larvae and foul-smelling meat were kept in a dark box in the basement near the furnace. Even without the aid of constant temperature and humidity chambers, a goodly supply of blowflies was usually to be had at any time. The only precautions taken were to observe the flies daily, chiefly to see that they had a sufficient quantity of fresh sugar water. Occasionally the cages were washed with water and the dead flies removed.

#### THE DARK ROOM AND APPARATUS

A large closet, 6.5 feet wide, 7.5 feet long, and 9.5 feet tall, in the writer's laboratory was converted into an experimental dark room (fig. 3) by building a ventilator (a) in the window, suspending from



the ceiling a 60-watt light (*b*) inside a white globe, and making the door (*e*) light proof.

The olfactometer (*d*) stood on a table (*e*) near the center of the room directly beneath the light, which was 4 feet above. The three bottles were connected with a glass Y tube (*f*), which was attached to a long glass tube running to the wall of the room. The latter tube was connected with a small brass pipe that penetrated the wall and ran to the blower pump (*g*) outside the dark room. As this one-tenth horsepower pump made considerable noise, it was mounted on large rubber stoppers inside a box that had a lid. The box rested on the top of a bookcase that stood in the laboratory against the wall of the dark room. A 40-watt ruby light (*h*), when used, was suspended 1 foot directly above the olfactometer. A centigrade thermometer (*i*), the scale of which was marked in fifths of degrees, was hung on the wall. An electric fan (*j*) was used only during the hottest days because its air current interfered with the responses of the flies.

#### THE OLFACTOMETER

The wooden olfactometer (fig. 4), the simplest and most satisfactory one yet devised by the writer, consisted of two essential parts—a specially constructed wooden box and a set of three bottles with their connecting tubes. The box (*a*), 12 inches square and 3 inches deep (inside dimensions), had wooden sides and bottom and a wire-screen top that opened on hinges and was fastened with hooks.

The box was supported on four wooden legs 8.75 inches long. At one side of the box there was a stopper (*b*) closing a hole 1 inch in diameter. This hole was used as an entrance and exit when the flies were being transferred to and from the box. On the same side of the box there was a door (*c*), 2.5 inches long and 2 inches wide, through which the dead flies were removed and the glass dish for sugar water was introduced. This dish (*d*), 2 inches in diameter and 1 inch deep, usually contained fresh sugar water on which floated a cork disk, having several round holes through which the flies took food while standing on the cork. The most specialized part of the olfactometer was the arrangement of two cups (*e*) which disseminated the odors entering the box. The cups were carved from disks of soft pine, 1 inch thick and 3.5 inches in diameter, and when finished were coated with hard paraffin that masked the pine odor. The cups (*f*) were 2.25 inches in diameter and 0.5 inch deep, and the rim stood three-sixteenths inch above the level of the disk. Galvanized-iron disks, each bearing 50 small nail holes, served as covers for the cups. The cover was fastened firmly to the rim with two small nails. The rim of the cup fitted snugly into a hole in the bottom of the box so that the

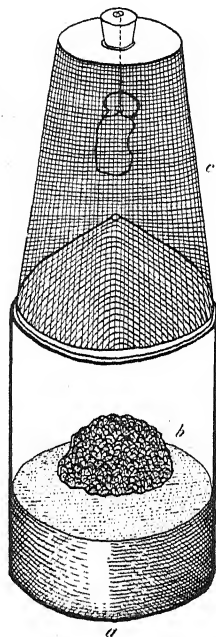


FIGURE 2.—Apparatus in which blowflies were reared and later were caught: *a*, Battery jar containing sand and Hamburg steak (*b*); *c*, flytrap from the top of which is suspended a piece of cotton wet with sugar water

perforated iron cover was flush with the inner surface of the bottom of the box. The cups were held in this hole by two rubber bands 1 inch wide, that were stretched the full width of the box on the under side. At the center of the bottom of the cup there was a hole just

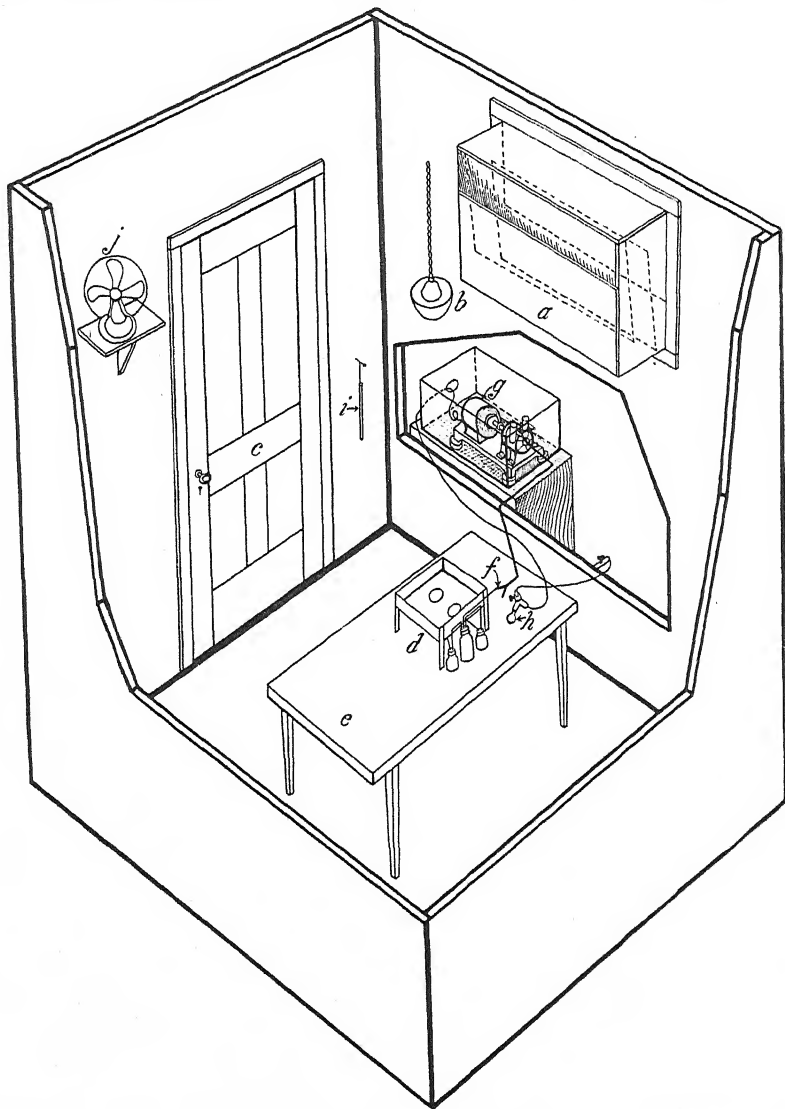


FIGURE 3.—Dark room and apparatus used in obtaining response of flies to odors: *a*, Ventilator; *b*, 60-watt light (not in correct position); *c*, door; *d*, olfactometer; *e*, table; *f*, glass Y tube; *g*, electric blower pump; *h*, 40-watt ruby light; *i*, centigrade thermometer; *j*, electric fan

large enough to admit the glass tube running from one of the smaller bottles.

The set of bottles consisted of one large bottle (fig. 4, *g*) of 200-cc capacity and two small ones (*h* and *i*) each of 100-cc capacity. Each bottle was closed with a No. 7 cork stopper that had been boiled in

hard paraffin. The stopper in the large bottle had three holes and each of the other stoppers had two. The glass tube (*j*), which connected bottle *h* with the nearer cup, was 7 inches long (not counting the two ends bent at right angles); while the corresponding tube (*k*), connecting bottle *i* with the farther cup, was 12 inches long. The tubes *l* and *m* were alike, each being 6 inches long. Tubes *n* and *o* were also alike, each being 4 inches long (not counting the two bent portions). The bore of each of the foregoing tubes was 3 mm. The length of tubes *p* and *q* was 6 inches each and the bore was 6 mm. The bore of the Y tube (*r*), of the glass tube (*s*), and of the brass tube which connected tube *s* with the blower pump was 8 mm. All of these tubes were securely united by short pieces of rubber tubing (*t*)

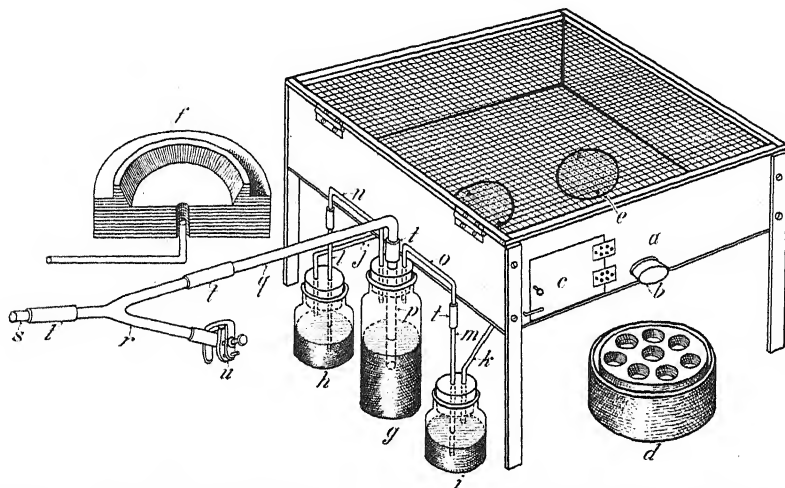


FIGURE 4.—Wooden olfactometer consisting of: *a*, Special wooden box in which flies were kept while being tested; *b*, stopper closing a hole used as an entrance and exit for flies; *c*, door of box through which dead flies were removed and through which sugar-water dish (*d*) was introduced when flies were not being tested; *e*, cup covered with perforated iron disk; *f*, portion of cup showing parts; *g*, large bottle; *h*, *i*, small bottles, each half full of liquid; *j*, *k*, tubes running from small bottles to cups; *l*, *m*, *n*, *o*, tubes connecting small bottles with large bottle; *p*, *q*, tubes connecting large bottle with Y tube (*r*), which unites with tube (*s*) running to blower pump; *t*, pieces of rubber tubing; *u*, clamp

in such a manner that practically none of the rubber was exposed to the air in the tube.

#### TRANSFER OF FLIES AND METHOD OF OPERATING THE OLFACTOMETER

During the preliminary stage of this work only one olfactometer was used for both species of blowflies, but subsequently an olfactometer was used for each species. The following procedure was usually adhered to closely. If the flies were on hand each series of tests was started with a set of newly emerged flies that had eaten little or no food. The number in each set usually ranged from 200 to 300. If the flies were to be transferred from a trap (fig. 2, *c*) to the box of an olfactometer, the trap was covered with a black cloth and the flies were then attracted by light through the round opening in the top of the trap and into the olfactometer box through the hole (fig. 4, *b*). If they were to be transferred from a cage (fig. 1), they were caught quickly in large vials and transferred to the box by means of light

and without the aid of a black cloth. They were handled quickly, without being touched with the fingers or tools, and for two or three weeks, or until the close of the experiments, they remained in the box, where they were usually fed lightly each night with fresh sugar water.

On the day after the flies were placed in the box of an olfactometer, the empty sugar-water dish was removed and the insects were carried to the dark room and placed directly beneath the light. (Fig. 3.) After the bottles had been half filled with tap water, the olfactometer was assembled and connected with the blower pump, which was well oiled with white petrolatum oil. This oil has no noticeable odor, but air passing through the pump had a faint machinelike odor, which was practically removed by forcing the air through the water in the large bottle. (Fig. 4, *g*.) A uniform flow of air was maintained by the use of a clamp (*u*) on a rubber tube attached to one fork of the Y tube. The number of bubbles counted in a small bottle was about 180 per minute. This weak air current could not be perceived by the fingers but it could be felt by placing one's cheek almost against the disk.

Before the pump was started the flies were fairly well distributed throughout the box, but just as soon as the humid air current entered the box those nearest the disk perceived it and went to the disks. This fact explains why this type of olfactometer is ideal for certain flies, particularly blowflies. So far, however, it has not been found satisfactory for the Colorado potato beetle (*Leptinotarsa decemlineata* Say), the Mexican bean beetle (*Epilachna corrupta* Muls.), or the codling moth (*Carpocapsa pomonella* L.). House flies responded readily to the humid air current, but, owing to their extreme restlessness, it was difficult to obtain consistent results. When attractive odors were being tested the response to weak air currents was not necessary, but when repellent odors were tested it was all important, for without it no results could be obtained. As the flies soon failed to respond to air currents or to odors carried by these currents, 10 counts were taken at 15-second intervals. The flies counted were those on the disks and those around the edges not more than one-fourth inch away. The counts were begun a few seconds after the air was turned on. The sum of the 10 counts for one disk was considered the result of a single test. A calculating machine was used for making the calculations.

The following data illustrate the preceding points and others not yet mentioned:

On March 13, 1931, there were about 300 females of *Lucilia* in the box. They had been fed lightly the night before, and at 9 a. m. the dish containing sugar water was removed. At 1 p. m., when the testing was begun, the temperature of the room was 23° C. and that of the water in the three bottles was 21°. The first control test gave the following figures, which represent the number of flies on each of the disks (fig. 4), as determined by counts made at 15-second intervals:

Humid air from bottle *h*—16+18+16+15+16+13+15+17+14+13=153.

Humid air from bottle *i*—18+16+13+13+13+14+17+16+15+12=147.

These figures show that the difference between any two counts on the same disk was usually not great. After bottles *h* and *i* had been interchanged, humid air from bottle *h* attracted 137 flies and that from bottle *i*, 117. In the first control test, bottle *h*=51 per cent attractive; in the second control test, bottle *h*=53.9 per cent attractive; average attractiveness, 52.4 per cent.

The water was removed from bottle *h* and an equal quantity of fermented granulated-sugar water was put in it. With the same procedure, the two tests gave the following results: Sugar water attracted 200 and 175 flies, respectively, and control water attracted 119 and 96 flies, respectively. In the first test of sugar water it was 62.7 per cent attractive; in the second test it was 64.6 per cent attractive; average attractiveness, 63.6 per cent.

Five days later a mixture of very putrid Hamburg steak and water was boiled hard for an hour and some of the boiled liquid was tested, the flies from the preceding test being used. The two tests gave the following results: Control water attracted 149 and 160 flies, respectively, and foul liquid attracted 50 and 60 flies, respectively. The repellency was obtained by calculating the attractiveness of the water as control. The first test of foul liquid was 74.9 per cent repellent; the second test, 72.7 per cent repellent: average repellency, 73.8 per cent.

After using about 300 males and females of *Calliphora* and testing other substances, it was ascertained that these flies, if fed lightly the night before, did not respond in large numbers; but if given neither food nor water overnight they responded in greater numbers, although the final percentages were not usually changed. A single test illustrates this point:

Water as control— $4+5+3+2+3+2+3+3+0+1=26$ .

Water from sour milk— $20+20+25+25+25+25+25+25+25+25=240$ .

The sour milk was 90.2 percent attractive.

When the flies were satiated with water or sugar water it was useless to carry on experiments. They did not then respond to most of the test liquids in sufficient numbers to give reliable results, but when certain much more attractive odors were used the factor of water satiation did not materially interfere with the responses expected. The plan finally adopted was to feed the flies lightly the night before the test and not to start the test until 10 or 11 a. m. This procedure was not at all necessary, however, when testing the odors of foul meat and a few other highly attractive materials.

Flies responding to the control liquid were in most instances accurately counted; but when responding to humid air or to odors in numbers more than 12 or 14, they were estimated, for it was impossible to count them, owing to their restlessness. Many times they were estimated, then counted, and the estimated number was nearly always smaller than the actual one.

It has already been shown that in running control tests the numbers of flies on the right and left disks did not differ materially. This was true only when the olfactometer was in perfect working order. If one cup or disk were scented or if a drop of odorous liquid were drawn into a cup, the numbers differed widely. The cups and disks were ordinarily made odorless by running air through them. Often they were removed and washed with water; but after foul odors had been tested, they were washed with alcohol and well aired. Before a series of tests was started the olfactometer was put in perfect working order and thereafter no liquid was permitted to come in contact with the cups or disks, which were cleaned and used in such a way that little or no error resulted from using the same cups repeatedly.

In a few tests the control response was actually 50 per cent. In most tests it was not above 52 or below 48 per cent, and the highest

recorded was 54.8 per cent. Responses above 50 per cent, not preceded by a plus or a minus sign, will hereafter be called attractive; while those above 50 per cent, preceded by a minus sign, will be called repellent. Nevertheless, in each test 2 or 3 per cent should be deducted as the probable error.

The flies, particularly the *Lucilia*, were sensitive to dry-air currents, but less so than to humid-air currents. Dry-air currents were not used as controls for odor responses because only odorous liquids were tested.

Flies were found sensitive to warm humid-air currents when cool humid air was used as a control. When there was only 2° or 3° difference in temperature between the water in the small bottles little or no difference in response was observed; but in one instance where there was a difference of 9° the warmer current was 74.4 per cent attractive. Similar results were obtained when dry currents and warm humid currents were tested. When humid currents were compared with dry currents the former were always the attractive ones. When humid air was forced through only one cup the response was as high as 85.8 per cent; the other 14.2 per cent of the flies just happened to be above the other cup, which was not a control and not connected with the other bottle.

After the foregoing results were obtained, the temperatures of the liquids in all three bottles were equalized by the use of cold and hot water and the thermometer brought from the dark room. The temperatures in most tests were near that of the laboratory.

#### DIFFERENCES IN RESPONSE OF MALE AND FEMALE BLOWFLIES

In order to obtain accurate results for comparative purposes several factors were considered during the preliminary stage of this work; however, since no long series of tests was specially planned and conducted in order to furnish definite information on all of these factors, the following data sufficed as a foundation for more thorough experimentation.

Of all the factors considered, the state of water satiation seemed to be the only one that interfered with the testing. This factor was easily controlled, as already stated, by giving the flies a small quantity of water or sugar water overnight. Other factors were considered, but no important differences in response were obtained that could be attributed to differences in age, in sex, or even in sexual maturity of the males and females tested.

Some of the results obtained between February 11 and March 11 varied considerably. To determine whether this variation was due to different proportions of males and females in the same box, the sexes of 599 *Lucilia* were separated on March 11 and 12, when the flies were 13 days old. Separation was easily effected by catching the flies in large vials, in which they were chilled, either out of doors or in an electric refrigerator. While their compound eyes were being observed, they were kept in cold water. In all, there were 219 males and 380 (or 63.4 per cent) females. Next, all the dead flies in the cage—47 females and 151 males—were examined. At the time of emergence, therefore, the females predominated slightly, constituting 53.6 per cent of the total number. The males were usually smaller and less hardy than the females, and died more quickly. On April 7, 574 *Calliphora*,

4 days old, were separated on the basis of sex, and 304, or 53.0 per cent, were found to be males.

The *Lucilia* were tested from March 12 to March 18 and the *Calliphora* from April 8 to April 14. Unfortunately, some of the materials tested, particularly the fermented ones, were slowly changing, but at that time of year the chemical changes taking place in them usually were not great. As not all the experimental conditions were exactly alike the figures in Table 1 should not be compared too closely. The chief purpose in submitting these figures is to show that there were few wide differences in response and that the averages for the males and females of the same species were practically the same.

TABLE 1.—*Olfactory responses of male and female blowflies to various substances*

Materials tested	Lucilia		Calliphora	
	Males	Females	Males	Females
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Control (water on each side).....	51.8	51.0	50.3	52.0
Fermented granulated-sugar water.....	56.1	64.6	69.3	74.6
Fermented granulated-sugar water and yeast.....	54.2	76.9	70.0	99.8
Fermented brown-sugar water.....	73.2	64.5	81.9	79.7
Fermented brown-sugar water and yeast.....	a 56.0	61.3	83.2	66.7
Fermented yeast mixture.....	83.9	85.2	86.7	70.3
Alcohol (about 6 per cent).....	a -51.7	52.4	74.0	77.5
Fresh milk.....	72.9	55.6	67.9	66.7
Water from clabbered milk.....	64.1	78.6	77.8	77.9
Fermented lactose mixture.....	60.1	61.3	93.0	92.3
Lactic acid (10.2 per cent).....	74.5	67.0	72.5	78.6
Fermented casein mixture.....	71.3	62.3	83.6	94.6
Formalin (1 cc of formaldehyde to 60 cc of water).....	74.0	61.7	61.5	70.0
Fermented molasses and yeast.....	54.5	60.7	74.5	60.9
Water from putrid Hamburg steak.....	84.6	82.3	89.4	90.5
Fermented molasses.....	60.9	66.0		
Fermented corn sirup.....	69.1	69.9		
Ammonia water (0.05 cc to 60 cc of water).....			66.7	71.0
Stale urine.....			76.1	64.6
Stale feces.....			80.0	63.7
Stale distillate from water and putrid steak.....			87.6	82.1
Skatol (water moderately scented).....			54.5	55.0
Average.....	64.5	65.9	75.0	72.9

a For negative numbers the complement was used in computing the average. e. g.,  $100 - 56 = 44$ .

## RESPONSE TO ODORS FROM FERMENTING AND PUTREFYING SUBSTANCES

In an attempt to obtain fundamental facts concerning the attraction of insects to odors from various fermenting and putrefying substances much experimental work was done. In these experiments the following materials were tested: 10 per cent solutions of granulated and brown sugars, with and without the addition of baker's yeast; various concentrations of carbon dioxide, alcohol, and acetic acid; milk and three of its constituents (lactic acid, lactose, and casein); putrid Hamburg steak, putrid eggs, human feces, human urine, ammonia, and skatol. The absence of accurate knowledge of the chemical composition of the various substances made it impossible to obtain more than a limited amount of definite information on the attractants concerned in the tests. Hence, as to this, only a brief summary is given in the present paper.

Sugar solutions not containing baker's yeast were attractive up to the seventeenth day, being most attractive (76.2 per cent) on the

fourth day. Between the eighteenth and twenty-fifth days (end of tests) they were repellent, being most so (72 per cent) on the last day they were tested. Sugar solutions containing baker's yeast were attractive only up to the twentieth hour, being most attractive (66.7 per cent) one hour after they were prepared. They were most repellent on the third day (93.7 per cent) and on the twenty-first day (94.6 per cent), but on the twelfth day they were only slightly repellent (55.9 per cent). Curves representing the attractiveness of these materials would therefore have two ascents and two descents. All concentrations of the carbon dioxide and acetic acid tested were repellent. All concentrations of the alcohol up to 6 per cent, inclusive, were attractive, but the higher concentrations were repellent. The 4 per cent concentration was most attractive (67.8 per cent), and the 20 per cent concentration most repellent (96.2 per cent). The evolution of carbon dioxide and the production of alcohol and acetic acid in the fermenting mixtures apparently had much to do with the responses obtained.

The only pertinent reference to the fermenting mixtures mentioned here is by Eyer and Rhodes (1, p. 702). They state:

Chemical analyses of codling moth (*Carpocapsa pomonella*) molasses baits during their periods of maximum attractiveness reveals their attractant value to be most closely associated with certain changes in the decomposition of the sugars which precedes the formation of alcohol and acetic acid and is first evidenced by a decrease in the glucose content of the baits. These phenomena which are probably attended by the formation of esters, the exact composition of each of which is as yet undetermined, are of primary importance. The formation of alcohol and the evolution of gas are important secondary factors, while the production of high yeast populations and the conversion of alcohol into acetic acid are only slightly attractive or actually repellent. Under New Mexico conditions, where the average mean daily temperature for the period during which the Codling moth adults are most active (May 1 to September 30) is 73.5° Fahrenheit, the effectiveness of molasses baits is actually decreased by adding yeast in commercial form while on the other hand it is materially increased and the period of attractiveness prolonged by the addition of certain preservatives, particularly Benzoate of Soda which delays fermentation.

Milk and its constituents—lactic acid, lactose, and casein—were always attractive. The more sour the milk the more attractive it was, the lactic acid in the milk probably being its chief attracting ingredient. Fermented casein and baker's yeast were among the best attractants, but putrid meat and putrid eggs were the most attractive. The responses to odors from such putrid materials were apparently caused by a "desire" to oviposit rather than to feed but, strange to say, the males responded as well to such odors as did the females. Although the sense of smell in blowflies is in many respects similar to that in human beings, the writer found it to be more acute than his own.

According to the average percentages (Table 1) obtained by testing fermented yeast mixture (81.5 per cent), fermented lactose mixture (76.7 per cent), fermented casein mixture (77.9 per cent), and water from putrid Hamburg steak (86.7 per cent) the attractiveness of the putrid steak appears to be little greater than that of each of the other three substances named. However, the difference is greater than it appears, for the total number of flies estimated to be on the disk is not a fair criterion by which to judge the attractiveness of foul-meat odors.

During the first three estimations nearly half of the flies in the box were attracted toward the left disk, but not more than 60 at a time



were recorded as being attracted. They piled upon one another on the disk two or three layers deep, and for about 3 inches around the margin they congregated, all headed toward the disk, waiting to climb upon the others. In a single test 395 flies responded to water from foul meat on the left while 85 responded to water as control on the right. The foul odor was, therefore, 82.3 per cent attractive. The number of flies responding on the control side was unusually large, and this accounts for the comparatively low percentage of attractiveness.

#### TESTS TO DETERMINE WHETHER BLOWFLIES SMELL WITH THEIR ANTENNAE

Before 1880 there was a controversy about the location of the olfactory organs in insects. In that year Hauser (3) apparently settled the matter by proving that the organs of smell are located in the antennae. In 1914 McIndoo (4, p. 333-341) convinced himself that honeybees do not smell with their antennae, but with widely scattered structures called olfactory pores. Von Frisch (2) in 1921 convinced most of the critics that honeybees do smell with their antennae. In 1924 Minnich (6) proved that nearly half of the olfactory organs of the cabbage butterfly (*Ascia rapae* L.) must be located elsewhere than in the antennae. With blowflies of the two larger genera, *Calliphora* and *Phormia*, and with the aid of the wooden olfactometer, the writer decided to close the controversy, if possible, so far as blowflies are concerned.

On January 1 and 2, 1932, 315 *Calliphora* emerged, 56.2 per cent being females. On January 4 and 5, the antennae or parts of antennae of 283 flies (126 males and 157 females) were pulled off, the following technic being used: About 30 flies at a time were caught in a large vial which was then placed in ice water. After the flies had become chilled the inactive ones were held between two fingers under a dissecting lens and the antennae were pulled off with fine-pointed forceps. The flies were then placed in the box of the olfactometer, where they were fed lightly over night and where they remained until they died. These mutilated flies could still fly well and responded normally to light and to odors, but they were comparatively inactive, both inside and outside the box, and were therefore easily caught with the fingers. They lived from 1 to 16 days, or an average of 8.2 days.

An excised antenna or part of one, when carefully examined under a high-power lens, was seen to have 1, 2, or 3 segments, but the third, or terminal segment, which bears the so-called olfactory hairs, was always present. Therefore all of the third segments were prevented from functioning when the insects with removed antennae were tested, but 17.1 per cent (18.7 per cent in males and 15.6 per cent in females) of the total number of second segments and 49.2 per cent (52.6 per cent in males and 45.9 per cent in females) of the total number of first segments remained on the heads.

Since there were not enough unmutated *Calliphora* for a second set of flies, the responses of the mutilated ones which were tested from January 6 to 14, 1932, were compared with those of a set of unmutated *Calliphora* tested in the summer of 1931. (Table 2.) Although the nine substances tested in 1931 and in 1932 were not exactly identical, the average responses (76.1 and 75.5 per cent) of the two sets of flies were practically the same.

TABLE 2.—*Responses of Calliphora with and without antennae to various odors*

Substance tested	Unmuti- lated flies	Flies with antennae pulled off
	<i>Per cent</i>	<i>Per cent</i>
Fresh milk.....	69.2	59.8
Sour milk.....	77.3	86.5
Lactic acid (10.2 per cent).....	76.2	84.5
Alcohol (4 per cent).....	77.6	74.8
Formalin (1 cc to 60 cc of water).....	65.7	60.0
Brown-sugar water.....	78.9	78.0
Water from putrid Hamburg steak.....	89.9	88.7
Stale urine.....	76.1	75.3
Stale distillate from putrid steak.....	74.5	72.3
Average.....	76.1	75.5

The next series of experiments, extending over a period of eight days, consisted of testing milk, brown-sugar water (10 per cent concentration), five concentrations of alcohol, and seven concentrations of lactic acid. The milk and sugar water were tested when fresh (Table 3) and then put in pint milk bottles. The bottles were covered with cheesecloth and placed in the laboratory. The concentrations of alcohol and lactic acid were prepared by using absolute alcohol, 85 per cent lactic acid (U. S. P.), and distilled water. The milk and sugar water were tested only once daily, but four tests for each concentration of alcohol and lactic acid were usually conducted. The average percentages of attractiveness of these substances are shown in Tables 3 to 5.

TABLE 3.—*Responses of Calliphora with and without antennae to odors from milk and brown-sugar water*

Age of material (days)	Milk		Brown-sugar water	
	Unmuti- lated flies	Flies with antennae pulled off	Unmuti- lated flies	Flies with antennae pulled off
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
0.....	69.2	59.8	64.1	64.3
1.....	77.3	71.4	67.3	66.3
2.....	72.5	74.4	67.4	67.3
3.....	75.7	-----	-----	70.3
4.....	73.3	75.4	78.9	78.0
6.....	67.8	73.1	72.1	70.2
7.....	66.1	68.4	-----	-----
8.....	62.8	60.0	71.7	-----
Average.....	70.6	68.9	70.2	69.4

Curves representing the foregoing responses of the unmutilated and mutilated flies closely follow each other. They and the average percentages given in Tables 2 to 5 show clearly that *Calliphora* do not smell with their antennae.

The experiments were repeated, using *Phormia*, and this time more care was exercised in pulling off the antennae. On January 13 and 14 more than 600 *Phormia* emerged, and on January 15 and 16 the antennae of 300 (113 males and 187 females) were removed under a binocular microscope. The mutilated insects were then placed in one box of the olfactometer, and 300 unmutilated flies, in approximately equal numbers of males and females, were placed in another

box. At each wound a tiny droplet of blood issued, but the flies apparently did not suffer and they appeared normal in nearly all respects. They were less active than usual, however, and were easily caught with the fingers. They mated readily and continued to lay eggs up to the time of their deaths. The mutilated flies began ovipositing two days later than the unmutilated flies and deposited only about one half as many egg masses.

TABLE 4.—Responses of *Calliphora* with and without antennae to odors from five concentrations of alcohol

Concentration of alcohol (per cent)	Unmutilated flies		Flies with antennae pulled off	
	Individual tests	Average for four tests	Individual tests	Average for four tests
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	59.8 60.8 58.6 62.0 62.5	60.30	56.8 65.0 63.9 59.4 61.1	61.30
2.....	72.3 68.7 59.5 77.6	65.75	63.4 61.5 68.7 67.8	63.70
4.....	68.9 69.7 71.8 55.3	72.00	73.8 73.1 74.8 60.5	72.40
6.....	61.7 60.6 63.4 55.4	60.25	66.1 63.6 60.7 51.8	62.70
8.....	53.4 51.7 a —52.0	52.12	53.8 56.2 50.0	52.95
Average.....	62.12 63.42 61.86 60.94	62.08	59.60 64.42 63.66 62.72	62.60

a Complement of this number was used in obtaining average.

The removed antennae were mounted in pairs in glycerin on two slides and the segments in each were counted. The third or terminal segment was always pulled off, the second segment was usually severed, but the minute first segment was often left attached to the head. Of the males, 38.0 per cent had been deprived of all 6 segments; 26.5 per cent of 5 segments; 24.8 per cent of 4 segments; 4.5 per cent of 3 segments; and 6.2 per cent of only 2 segments. Of the females, 40.7 per cent had been deprived of all 6 segments; 23.0 per cent of 5 segments; 25.0 per cent of 4 segments; 7.5 per cent of 3 segments; and only 3.8 per cent of 2 segments. An average of 39.3 per cent of both males and females had been deprived of all 6 segments; 24.7 per cent of 5 segments; 24.9 per cent of 4 segments; 6.0 per cent of 3 segments; and only 5.0 per cent of 2 segments.

As each box contained a larger number of flies, it was impossible to obtain complete records of individuals; therefore those that had been deprived of all or nearly all of their antennal segments might not have responded at all. Having failed to obtain satisfactory records by using only 1, 2, or 3 mutilated *Calliphora* at a time in the box, 7 active unmutilated *Phormia* were placed in one box and 7 active

mutilated *Phormia* in another, each fly in a box being distinctively marked with white ink for easy identification. On a form used in tabulating the results the symbol for each fly indicated the sex of the insect and the part or parts of its anatomy that were marked. The following abbreviations were used: t for thorax; r for right wing; l for left wing; and a for abdomen. The unmutilated flies were, therefore, given the following symbols: t♂, r♀, l♂, rl♂, tr♀, tl♂, and a♀. The symbols of the mutilated flies were t♀, r♀, l♂, rl♂, tr♀, tl♂, and a♀. The marked flies were among a large number of unmarked flies.

TABLE 5.—Responses of *Calliphora* with and without antennae to odors from seven concentrations of lactic acid

Concentration of lactic acid (per cent)	Unmutilated flies	Flies with antennae pulled off	
		Individual tests	Average for four tests
	Per cent	Per cent	Per cent
1.7.....	53.6	54.4 60.0 59.1 60.6 61.4	58.5
3.4.....	59.5	63.7 61.6 58.6 67.0	61.3
6.8.....	72.7	67.0 71.0 69.3 71.9	68.6
10.2.....	76.2	72.1 76.6 84.5 60.0	76.3
13.6.....	73.7	73.7 73.3 67.8 58.1	68.7
17.0.....	60.0	62.7 64.2 67.2 51.3	63.0
25.5.....	<sup>a</sup> —53.6	58.1 57.0 57.9	56.1
Average.....	63.15	60.58 65.32 66.11 66.55	64.64

<sup>a</sup> Complement of this number was used in obtaining average.

Fresh brown-sugar water (10 per cent concentration), fresh milk, concentrations of alcohol and lactic acid, and mixtures containing baker's yeast cake (3 g in 250 cc of water), and purified casein (3 g in 250 cc of water) were prepared. Pint milk bottles containing the sugar water, milk, yeast mixture, and casein mixture were covered with cheesecloth and placed in the laboratory. The alcohol and lactic acid were kept in well-stoppered bottles. The tests were run from January 18 to 26.

Tables 6 to 8 show the average percentages of attractiveness of the substances. The general average is, for unmutilated flies, 66.6; for mutilated flies, 67.4. Again, it is clearly shown that blowflies do not smell with their antennae.

TABLE 6.—Responses of *Phormia* with and without antennae to odors from brown-sugar water, milk, yeast mixture, and casein mixture

Age of material (days)	Brown-sugar water		Milk		Yeast mixture		Casein mixture	
	Unmutilated flies	Flies with antennae pulled off	Unmutilated flies	Flies with antennae pulled off	Unmutilated flies	Flies with antennae pulled off	Unmutilated flies	Flies with antennae pulled off
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
0.....	64.3	65.1	59.3	64.3	65.2	70.4	63.6	67.1
1.....	68.0	72.1	66.2	69.8	65.9	68.5	69.0	67.2
2.....	68.2	69.6	66.0	71.7	68.4	71.4	68.5	72.5
3.....	69.3	69.8	78.0	72.7	67.4	70.4	69.7	67.1
4.....	73.6	75.3	79.8	76.1	77.4	73.8	76.4	80.3
5.....	65.4	65.2	66.0	68.4	72.0	65.5	77.7	75.8
7.....	57.6	60.2	62.3	60.2	71.2	75.4	81.2	74.3
8.....	57.4	60.0	59.2	60.9	72.2	76.1	80.8	81.6
Average.....	65.5	67.2	67.1	68.0	70.0	71.4	73.4	73.2

TABLE 7.—Responses of *Phormia* with and without antennae to odors from five concentrations of alcohol

Concentration of alcohol (per cent)	Unmutilated flies		Flies with antennae pulled off	
	Individual tests	Average for 4 tests	Individual tests	Average for 4 tests
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1.....	57.3 59.4 58.6 58.2 62.6	58.375	56.6 59.5 58.5 58.8 62.9	58.35
2.....	61.8 66.1 64.9 73.7		62.9 67.3 63.5 74.4	
4.....	65.4 65.9 66.2 63.4		65.4 70.0 66.1 64.1	
6.....	62.9 62.5 60.0 a 50.7		62.5 63.5 55.8 a 50.9	
8.....	b-56.9 b-58.5 b-54.6		b-51.5 b-50.6 b-50.0	
Average.....	61.54 58.72 58.92 58.94	59.53	61.78 59.76 61.74 57.04	60.08

<sup>a</sup> Complement of this number was used in obtaining average, 4 tests, but the percentage as it stands was used in obtaining average, first test, all concentrations.

<sup>b</sup> Percentage as given was used in obtaining average concentration 8, but its complement in obtaining averages of the second, third, and fourth tests, all concentrations.

On the basis of equal response by all the flies in the box the data for 4 days showed more than proportional response by the marked flies and the mutilated flies responded slightly better than did the unmutilated ones. The females responded almost as often as the males. The total number of responses of each of the 14 marked flies ranged from 3 to 24, with an average of 13.9. Considering all of the 14 marked flies and the 7 substances tested, water (as a control) offered 50 per cent of all the chances to respond, while each of the other 6 materials offered only 8.3 per cent. However, the following

data clearly show that the number of responses were not so divided. Water induced only 21.6 per cent of all responses; brown-sugar water, 9.8 per cent; casein solution, 9.3 per cent; lactic acid, 19.6 per cent; yeast mixture, 9.8 per cent; alcohol, 10.8 per cent; and milk, 19.1 per cent. Of the 7 mutilated flies alone, water induced only 18.7 (and not 50) per cent of all the responses; brown-sugar water, 5.6 per cent; casein mixture, 7.5 per cent; lactic acid, 18.7 per cent; yeast mixture, 13.1 per cent; alcohol, 14.0 per cent; and milk, 22.4 per cent. The total number of responses to all of the 7 materials tested is as follows: t♀, 8; r♀, 16; l♂, 16; rl♂, 20; tr♀, 24; tl♂, 15; and a♀, 8. When these 7 mutilated flies had died, their heads were examined to determine how many antennal segments remained attached. This was done by teasing out the "stumps" of the antennae with needles under a binocular microscope. The following results were noted: tl♂ bore no antennal segments; r♀, l♂, and rl♂ each bore one segment; t♀ and tr♀ each bore two first segments; and a♀ bore two first segments and one second segment. The 7 mutilated flies lived from 11 to 58 days, or an average of 31.6 days, and the 7 unmutilated flies lived from 1 to 58 days, or an average of 26 days. The 300 mutilated flies lived from 1 to 59 days, or an average of 15.9 days; the 300 unmutilated flies lived from 1 to 60 days, or an average of 18.2 days.

TABLE 8.—Responses of *Phormia* with and without antennae to odors from seven concentrations of lactic acid

Concentration of lactic acid (per cent)	Unmutilated flies		Flies with antennae pulled off	
	Individual tests	Average for 4 tests	Individual tests	Average for 4 tests
	Per cent	Per cent	Per cent	Per cent
1.7-----	<div>55.8 58.2 59.1 57.6 64.7</div>	57.675	<div>59.1 58.3 58.1 58.0 65.4</div>	58.375
3.4-----	<div>59.6 58.6 65.7 69.0 62.6</div>	62.15	<div>56.9 62.8 69.0 73.8 68.6</div>	63.525
6.8-----	<div>63.5 76.9 69.8 66.7 68.4</div>	68.0	<div>66.0 70.8 69.3 70.7 76.5</div>	68.10
10.2-----	<div>66.7 72.6 66.7 60.0 62.0</div>	69.375	<div>72.5 65.5 67.5 70.1 63.8</div>	71.825
13.6-----	<div>74.8 62.1 59.0 62.8 66.3</div>	65.875	<div>62.7 63.1 62.2 59.1 59.2</div>	68.90
17.0-----	<div>60.8 58.9 60.6 68.7</div>	62.55	<div>61.9 58.8</div>	62.95
25.5-----		62.25		59.75
Average-----	<div>64.13 60.71 62.14 68.94</div>	63.98	<div>66.35 62.93 64.01 65.80</div>	64.77

Some of the experiments were again repeated, using *Calliphora*. On January 18 about 140 *Calliphora* emerged and on January 25 and

26 the antennae of 134 of them were pulled off. This time the insects were not chilled by using ice water and a greater effort than ever was made to pull off all the segments by seizing, if possible, the minute first segments with the needle-pointed forceps. These operations were fatal to many of the flies, 93 of them having died by January 27, when the survivors (13 males and 28 females) were tested. An examination of the heads of the dead flies showed that 41.4 per cent of them had been deprived of all six antennal segments. On the heads of the others 40.2 per cent of the first segments and 21.9 per cent of the second segments remained, but no third segments.

On January 27, between 12.30 and 1.20 p. m., 10 males and 18 females, the most active of the surviving flies, were subjected to another operation. This time, in order to eliminate the possibility that the remaining antennal segments or the exposed antennal nerves might still receive olfactory stimuli, tiny droplets of glue were placed on the "stumps" of the antennae, and again the flies were tested. (Table 9.) When these flies had died, it was observed that the glue remained where placed and only 37.5 per cent of the first segments were present on the heads. The 41 tested flies lived from 1 to 14 days, or an average of 3.8 days. The flies with glue on their heads lived an average of 4.2 days. The data in Table 9 again clearly show that the antennae of blowflies do not carry the olfactory organs.

TABLE 9.—*Responses of Calliphora with and without antennae to various odors*

Substance tested	Unmutilated flies	Flies with antennae pulled off	Flies with antennae pulled off and stumps of antennae covered with glue
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Brown-sugar water (9 days old)-----	71.7	74.1	75
Milk (9 days old)-----	62.8	69.2	69
Lactic acid (10.2 per cent)-----	76.2	74.2	76
Yeast mixture (9 days old)-----	78.5	75.9	75
Casein mixture (9 days old)-----	89.1	81.2	75
Average-----	75.7	74.9	74

To obtain further information on this subject a careful study of the antennae of blowflies was made by using live antennae, caustic potash preparations, and many sections. The so-called olfactory hairs, lying in pits and pocket-shaped cavities in the third segments, are very numerous but they were not found elsewhere on the antennae. The first and second segments contain no sense organs of any kind, except the Johnston's organs in the second segment. These organs have no connection with the exterior and have never been regarded as olfactory organs. Furthermore, it was observed that wounds on the mutilated antennae soon healed, thereby preventing the air from entering at the places of injury.

#### SUMMARY

In the hope of obtaining fundamental facts concerning the attraction of insects to odors from fermenting and putrefying substances, much experimental work was done with three species of blowflies, but little conclusive information was obtained as to the definite attractant.

For this study large numbers of these blowflies (*Calliphora erythrocephala* Meig., *Lucilia sericata* Meig., and *Phormia regina* Meig.) of known ages were successfully reared in both summer and winter, without the aid of constant temperature and humidity chambers. The males and females, shortly after emergence, were about equal in number, but as the males died more quickly, the flies tested usually consisted of more females than males.

The olfactometer used was the simplest and most satisfactory one yet devised by the writer for work with blowflies. It consisted essentially of a wooden box having a wire-screen top, and of a set of three bottles, one large and the other two small. The bottles were connected on the left with an electric blower pump and on the right by means of glass tubes with two specially constructed cups that fitted into the bottom of the box. The large bottle and one small bottle were half filled with water, and the other small bottle was half filled with the liquid to be tested as an attractant or repellent. The pump forced humid air and odors from the bottles through the tubes and into the cups, and these were diffused in the box through the perforated covers on the cups. The key to success in this investigation was the ready response of blowflies to humid air currents; therefore, humid air, not bearing odors, from one cup served as a control, while humid air bearing odors from the other cup served as the attractant or repellent. The flies lived in the box usually two or three weeks or until the testing was finished. All the testing was done by diffused light in a dark room.

With the aid of the olfactometer it was first determined that the responses of the males and females did not usually differ widely and that the average responses of the males and females of the same species were practically the same.

When fermenting sugar solutions were tested during a period of 25 days, it was determined that solutions not containing baker's yeast were largely attractive, whereas solutions containing baker's yeast were repellent during nearly all of the 25-day period. Tests with carbon dioxide, alcohol, and acetic acid indicated that these substances in the fermenting mixtures had much to do with the responses obtained.

The responses to milk and three of its constituents—lactic acid, lactose, and casein—indicated that they were always attractive. Fermented casein and baker's yeast were among the best attractants found, but putrid meat and putrid eggs were the most attractive.

In order to determine whether or not blowflies smell with their antennae, three series of experiments were conducted. In the first series the antennae, or parts of antennae, of 283 *Calliphora* were pulled off so that all of the third or terminal segments, which bear the so-called olfactory hairs, were removed. Nine substances, including fresh and fermented milk and brown-sugar water, five concentrations of alcohol, and seven concentrations of lactic acid, were tested. The general average responses, expressed as percentages, obtained were: Unmutilated flies, 68.4; mutilated flies, 68.2.

In the second series of experiments the antennae of 300 *Phormia* were pulled off. For a period of 8 days brown-sugar water, milk, yeast mixture, and casein mixture were tested. Five concentrations of alcohol and seven concentrations of lactic acid were also tested. The general average responses were—unmutilated flies, 66.6, and mutilated flies, 67.4.



In the third series of experiments the antennae of 134 *Calliphora* were pulled off, and after the mutilated flies had been tested, the stumps of their antennae were covered with glue. The average responses to five substances were as follows: Unmutilated flies, 75.7; mutilated flies, 74.9; and flies with glue on the stumps of their antennae, 74.0.

From the preceding averages it is concluded that the antennae of blowflies do not bear the olfactory organs.

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# PERFECTING A STAND-DENSITY INDEX FOR EVEN-AGED FORESTS<sup>1</sup>

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## INTRODUCTION

An adequate expression for density of stocking in even-aged forests has long been sought by foresters. Comparison of total basal area of the stand with yield-table values of basal area for the same age and site quality has been the usual method of evaluating stand density. Other methods have been proposed, but none has given results good enough to warrant general adoption or displacement of the basal-area method. It is the purpose of this paper to present a stand-density index which does not require a yield table and which is not affected by possible errors in shape of the *total basal area-age* curve. This stand-density index, based on the relationship between number of trees per acre and their average diameter, is premised on the characteristic distribution of tree sizes in even-aged stands.

It is a well-established fact that in any given stand a curve showing the relative (percentile) frequency of occurrence of the various tree sizes (diameters) has a characteristic form, often approximating that of the "normal frequency curve" or "normal curve of error" (1, 2, 3, 4, 6, 7).<sup>2</sup>

## STATISTICAL BASIS

This frequency-curve form may differ with species; the departures from normal may embody positive or negative skewness (6), or a logarithmic form may be assumed (4). Within a given species, however, stands of all ages on all sites have essentially the same characteristic frequency-curve form (4, 7, 9).

The form of the frequency curve may be described by several statistical measures, those used most commonly being the average (or mean) diameter and the standard deviation, with the coefficients of asymmetry and of excess less generally used (6).

For any one general form of frequency curve, as applied to diameter distribution in even-aged stands, the percentile frequency curve for a specific stand is described primarily by the average diameter. The standard deviation and coefficients of asymmetry and of excess provide a further description of the curve, but since they are correlated with the average diameter they are of secondary importance. It is thus possible to a certain extent to describe the relative diameter distribution of a stand by average diameter alone.

## COMPARISON OF STAND DENSITIES

The concept of stand description is useful in comparing the density of stocking of various stands. Two stands of the same description

<sup>1</sup> Received for publication July 14, 1932; issued May 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 637.

(same average diameter and, by implied correlation, the same standard deviation, etc.) have the same relative distribution of diameters but may differ as to total number of trees per unit area. Obviously, the stand with the greater number of trees is the better stocked or denser stand of the pair and their relative stand densities are directly proportional to the number of trees on them. The number of trees per acre of one stand may be expressed as a percentage of the number of trees of the other; this percentage will indicate the relative stand densities. If data for a sufficient number of stands of the same description (average diameter) are obtained, the stand with most trees per acre can be considered as having 100 per cent stand density. If all other stands are referred to this one, the density of each can be expressed as a percentage of full density.

### THE REFERENCE CURVE

The number of trees per acre for full density varies, however, with the average diameter of the stand. Stands of small average diameter have a large number of trees, while stands of large average diameter have relatively few. To determine the density of stands of all descriptions, it is necessary to have a curve showing the number of trees per acre at full density for all average diameters.

This curve of maximum number of trees per acre over average diameter when plotted on ordinary cross-section paper is concave upwards, falling rapidly in the small diameters and flattening as the larger diameters are reached. When plotted on logarithmic cross-section paper, this curve assumes a straight-line form. For many species the slope of this logarithmic straight-line graph is constant, but its elevation differs with species. This curve is represented by the equation,

$$\log N = -1.605 \log D + k$$

in which  $N$  is the number of trees per acre,  $D$  is their average diameter (by basal area), and  $k$  is a constant varying with species. When  $k$  is 4.605 the curve passes through the point representing 10 inches average diameter, 1,000 trees per acre, as shown in Figure 1 (solid line). This curve will be referred to hereafter as the "reference curve."

### CONFORMITY TO THE REFERENCE CURVES

In Figure 2 is shown a series of California red fir (*Abies magnifica* A. Murray) yield-plot values as collected by Schumacher (9). The reference curve is a very good fit for the maximum values. Similarly, in Figure 3, a curve parallel to the reference curve but passing through 10 inches, 830 trees, expresses the maxima for yield-plot data for white fir (*A. concolor* Lindl. and Gord.).

Other species also conform to the reference curve. Curves parallel to the reference curve are fitted to yield-plot values for the mixed conifer types in California (measured by Dunning, Show, and others) in Figure 4, A, to second-growth Douglas fir (*Pseudotsuga taxifolia* (LaM.) Britt.) in Oregon and Washington (measured by McArdle) in Figure 4, B, and to second-growth Douglas fir in California (measured by Schumacher) in Figure 4, C.

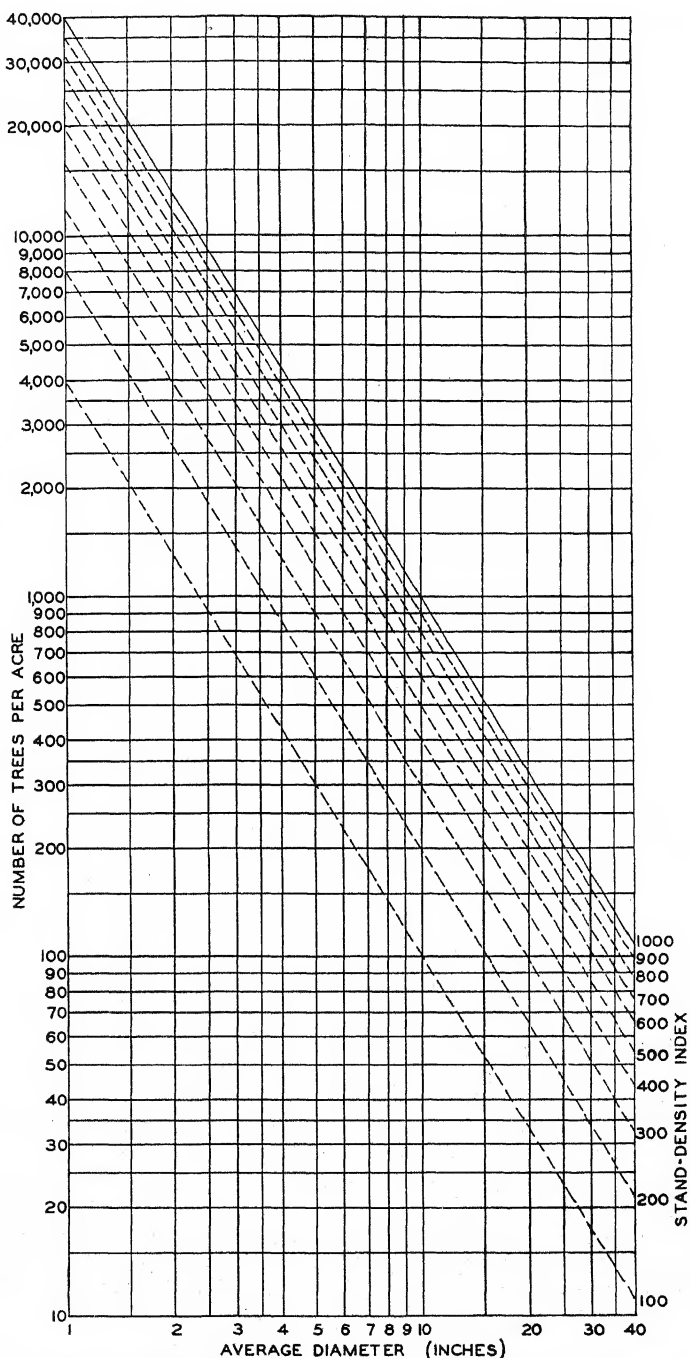


FIGURE 1.—Reference curve (solid line). The stand-density index of each of the broken-line parallel curves is the number of trees indicated by each at 10 inches average diameter

In Figure 5 data are shown for plantations of *Eucalyptus globulus* Labill. (5), for second-growth ponderosa pine (*Pinus ponderosa* Laws.) as measured by Gallaher, and for second-growth redwood (*Sequoia sempervirens* (Lamb.) Endl.) as measured by D. Bruce. Most of the eucalyptus plantations (fig. 5, A) were widely spaced and

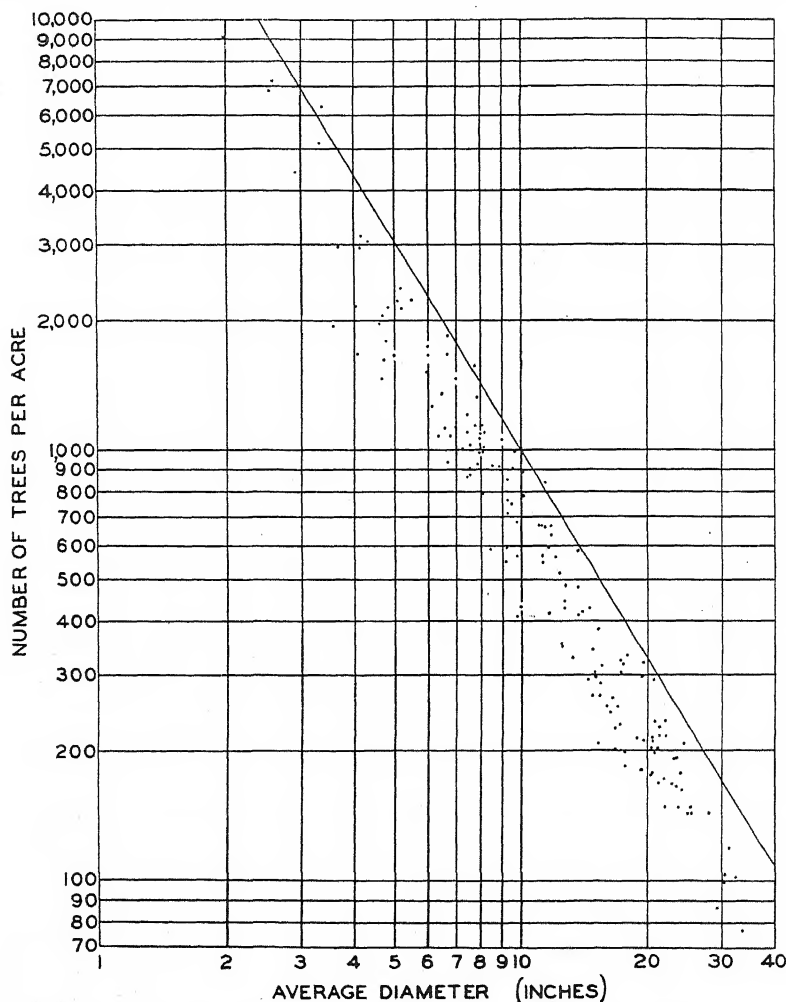


FIGURE 2.—Number of trees—average diameter relation for red fir, with reference curve defining the maxima

very young. They were, therefore, much understocked when measured. The maximum points to which the curve (parallel to the reference curve) has been fitted, represent the few closely spaced or older plantations.

The data for ponderosa pine (fig. 5, C) were taken to represent "overstocked" stands. Although these data cover only a small range in average diameter, they fit the curve of standard slope quite well.

The maximum curve for redwood (fig. 5, B) is less well defined. The distribution of plots by average diameter is poor, however, and additional data below 10 inches and above 20 inches are necessary to establish conformity or nonconformity with the reference curve.

In each of the preceding groups of data, the curve has been fitted to represent the maximum values rather than the average. Ordi-

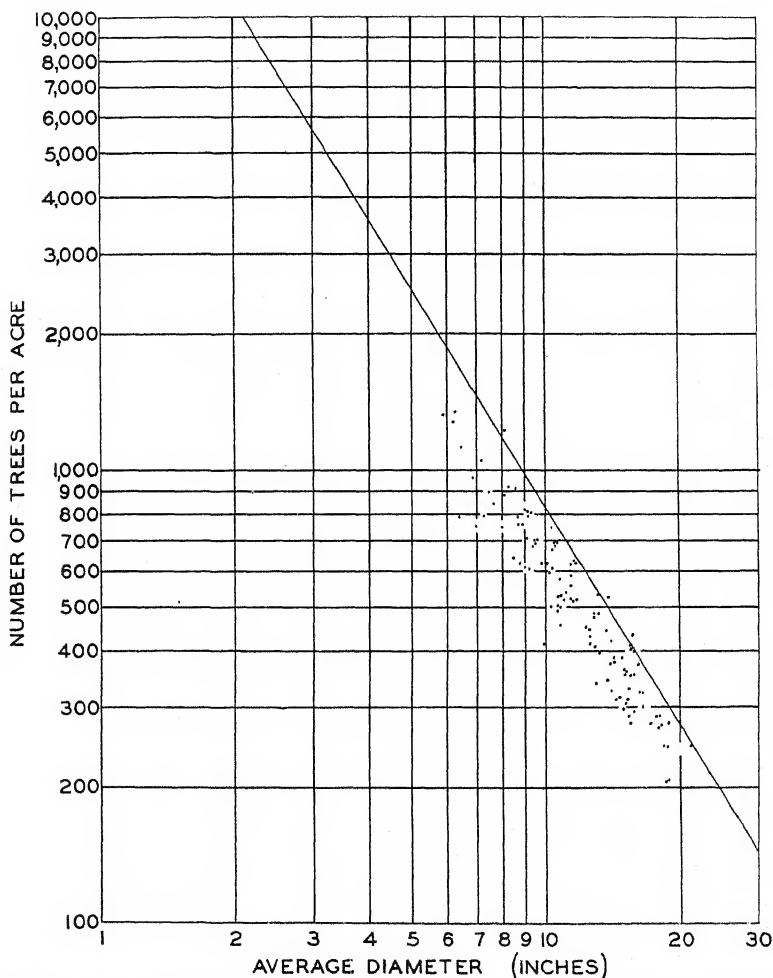


FIGURE 3.—The maximum stand-density index for white fir is 830, as shown by the curve defining the maxima and paralleling the reference curve

narily the average values of any group of data will define a curve better than the maxima, especially if the conditions of random sampling are met. In yield studies, however, biased sampling, as to stand density, is the rule. Plots are selected for maximum stocking and poorly stocked plots are not desired, but in some circumstances normality standards must be lowered to secure adequate representation of stands of certain ages or site quality. This is usually neces-

sary with the larger, older stands; since they are more mature, many of the better stands are cut, leaving only the poorly stocked ones which are less desirable commercially. The result of such a selection would be a lowering of average stand density for large stands, which would lower the large-diameter end of the number of trees—average diameter curve. This distortion is avoided by fitting to the maxima. would lower the large-diameter end of the number of trees—average diameter curve. This distortion is avoided by fitting to the maxima.

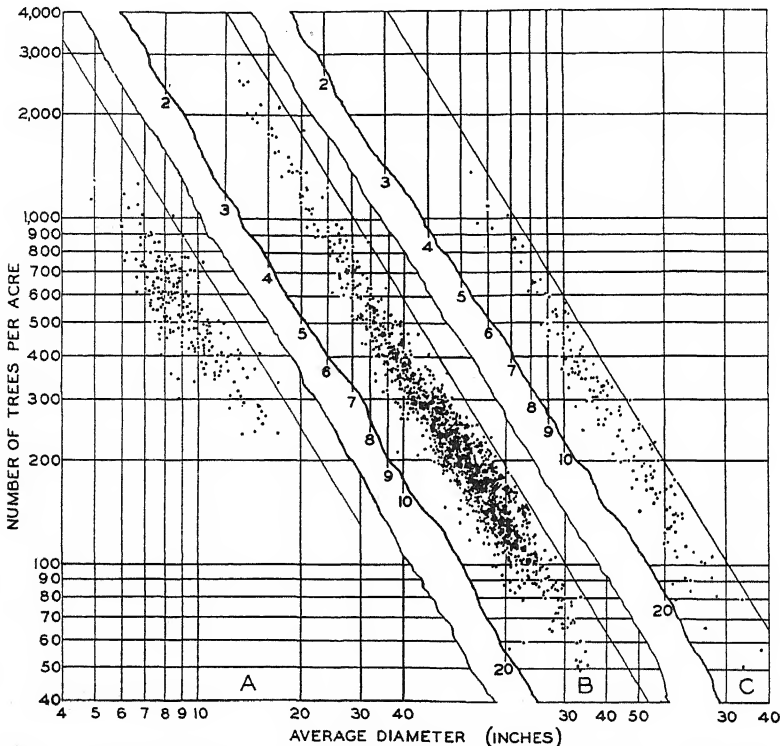


FIGURE 4.—Maxima curves for: A, Mixed conifer stands in California; B, Douglas fir in Washington and Oregon; C, Douglas fir in northern California. Note that the maximum stand-density index is almost identical (approximately 595) for both groups of Douglas fir

Where random sampling is secured, as in strip cruises, fitting to average points is more satisfactory. Figure 6 represents such a case, the data resulting from a strip cruise by G. H. Barnes in second-growth lodgepole pine in British Columbia (1a). The numbers in Figure 6 indicate the number of 1-acre plots represented by each average point. The solid line is parallel to the reference curve; the broken (straight) line corresponds to the curve fitted by Barnes on semilogarithmic paper.

In all the above examples, the reference curve, or one parallel thereto, has fitted reasonably well.<sup>3</sup> This is also true for longleaf pine (fig. 7, C) and for loblolly pine (fig. 7, B), based on yield-study plots. In Figure 7, A, showing measurements at 5-year intervals of

<sup>3</sup> This is also true for southern white cedar (*Chamaecyparis thyoides* (L.) B. & P.) and for northern white pine (*Pinus strobus* L.), not illustrated.



two permanent sample plots in loblolly pine, the same slope is indicated. The slope for slash pine, Figure 7, D, is slightly steeper than for the reference curve (broken line), although it is none too well defined. For shortleaf pine, however, the slope is definitely steeper. (Fig. 7, E.)

This possible nonconformity of slash pine, and the definite nonconformity of shortleaf pine, may be due, perhaps, to the influence of fire. Although unburned plots were sought for the yield study, few of them

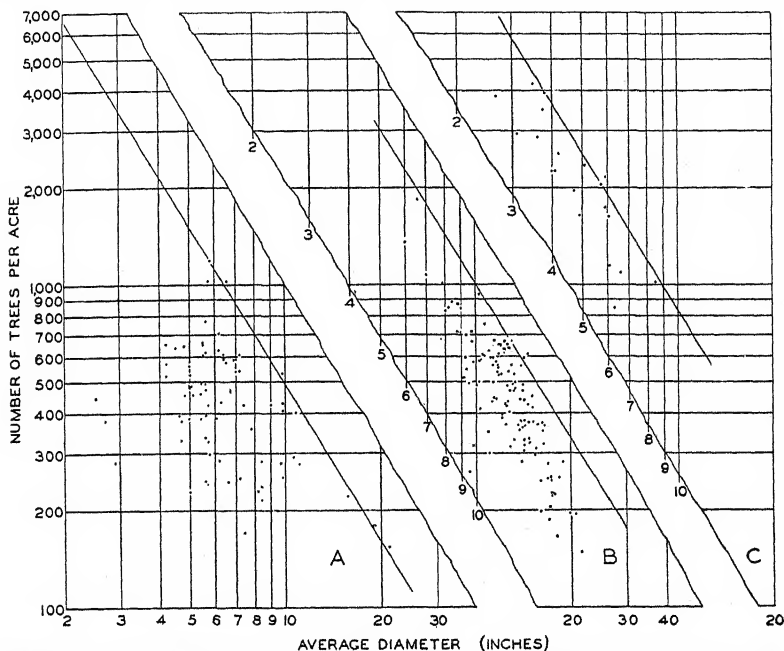


FIGURE 5.—Curves, parallel to the reference curves, for: A, Eucalyptus (in plantations); B, redwood; C, ponderosa pine

were entirely free of fire damage, and the chances of fire injury were obviously greater in the older, larger stands.

#### EFFECT OF AGE AND SITE QUALITY

Before accepting this relationship of number of trees per acre to average diameter as an index to stocking, it is necessary to examine into the possible effect of age and site quality. Multiple linear correlations were computed for several species with number of trees per acre in a percentage of the maximum curve as the dependent variable, and with height of average dominant tree of the stand and total age of stand as independent variables. Age and dominant height were used in preference to site index, since the latter would be influenced by any improper shaping of the site-index curves. The regression equations, with the statistical measures, are given in Table 1.

TABLE 1.—Regression equations and statistical measures resulting from multiple linear correlations of trees per acre, dominant height, and total age of stand

Species	Regression equation	Alienation coefficient	Correlation coefficient	Ratio of correlation coefficient to probable error
Douglas fir (in California).....	Percentage number of trees = $-0.067$ age + $0.011$ dominant height + $51.075$ .	0.990	$0.141 \pm 0.0501$	2.81
White fir.....	Percentage number trees = $0.006$ age + $0.095$ dominant height + $56.491$ .	.978	$.208 \pm .071$	2.93
Red fir.....	Percentage number trees = $0.229$ age - $0.194$ dominant height + $61.98$ .	.968	$.072 \pm .049$	1.47

For Douglas fir and white fir, the regression coefficients are very small. For red fir they are considerably larger, but the correlation coefficient is the smallest (0.072) and least significant (correlation coefficient only 1.47 times its probable error). For each species the correlation coefficient is small and insignificant, since it does not exceed three times its probable error.

Apparently, there is no significant or appreciable correlation between age or site quality and the number of trees per acre for a given average diameter. It is, then, safe to use the *number of trees—average diameter* curve as a standard to which similar values of an individual stand may be compared to determine its density of stocking.

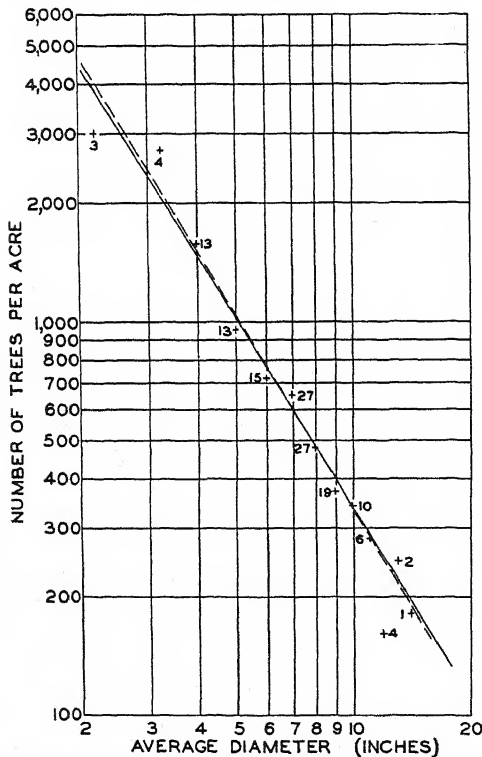


FIGURE 6.—Curve (solid line), parallel to reference curve, fitted satisfactorily to points based on strip cruises in lodgepole pine stands in British Columbia; the broken line corresponds to the curve fitted by Barnes on semilogarithmic paper

#### PERCENTAGE STOCKING

The numerical expression of density of stocking may take two forms. The obvious way to express this measure is to establish the position of the *number of trees—average diameter* curve for maximum or 100 per cent stocking for a given species and to express individual stand values as a percentage of this maximum curve. The value thus derived may be termed the "percentage stocking." This is a simple, usable measure, but has the disadvantage of requiring prior determination of the maximum curve. Comparison of one species with another is less simple, because of differences in maxima.

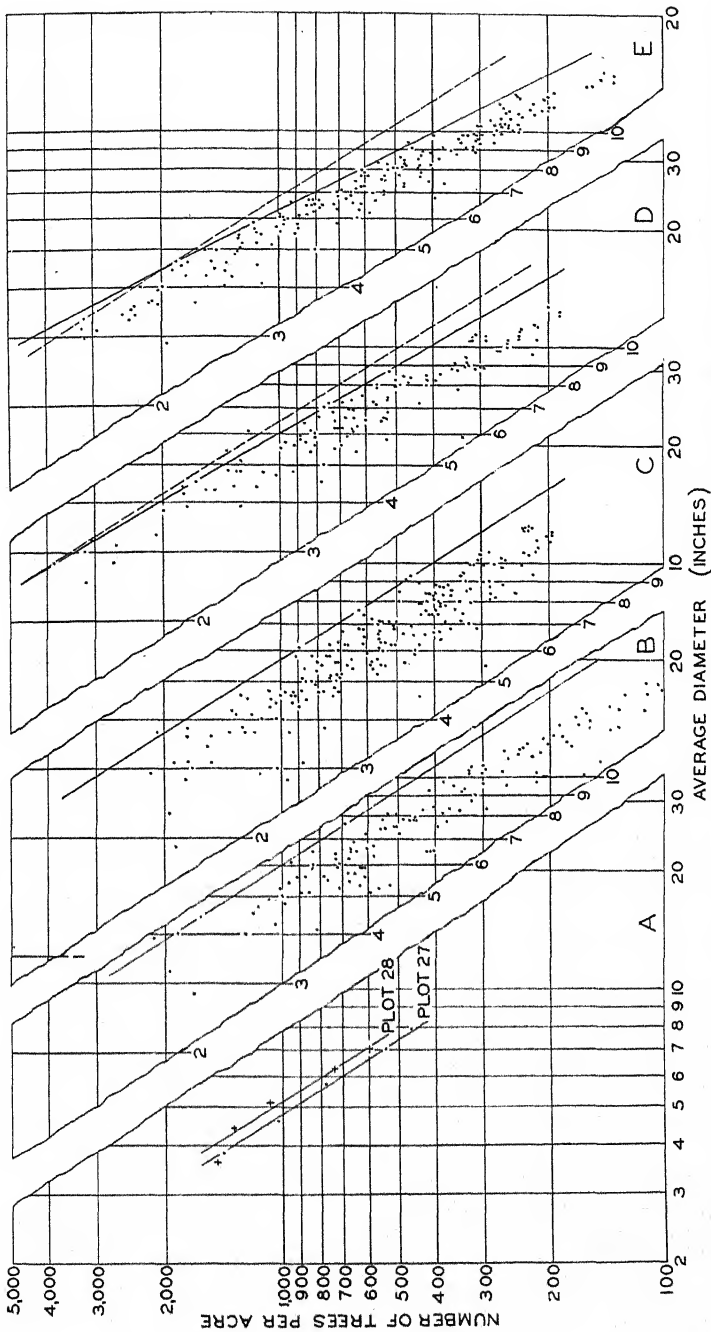


FIGURE 7.—Agreements with and divergences from the reference curve in different species. Parallelism is found in (A) loblolly pine at 5-year intervals, on permanent plots, (B) temporary yield-plot data for loblolly pine, and (C) longleaf pine, divergence is noted to a slight degree in (D, dotted line) slash pine, and more definitely in (E, dotted line) shortleaf pine

## STAND-DENSITY INDEX

A second expression for density, which may be termed the "stand-density index," permits direct comparison between species in addition to expressing varying stand densities within a species. Using the reference curve as the basis for all species for which the slope is the same as that of the reference curve,<sup>4</sup> the number of trees per acre in a given stand is plotted on a graph similar to Figure 1 (with the broken-line curves omitted) and a line is drawn through this point, parallel to the reference curve, until it intersects the 10.0 inch ordinate. The number of trees at this point of intersection is taken as the stand-density index.<sup>5</sup> Since the reference curve passes through 1,000 trees per acre at 10.0 inches, this value is ten times the percentage relation between the stand and the reference curve.

In practice, determination of the 10-inch intercept value by means of the parallel line as described above is not necessary. To Figure 1 has been added a series of broken-line curves parallel to the reference curve and intersecting the 10-inch ordinate at 100, 200, 300 trees, etc., and so labeled. When the average diameter and number of trees per acre of a stand are plotted on this graph, the stand-density index (to the nearest 50 trees per acre) is given by the parallel line nearest the plotted point. By interpolation between curves, stand-density index may be read easily to the nearest 10 trees per acre.

Stand-density index can also be expressed as a percentage of the reference curve. It is deemed more desirable, however, to use the number of trees as the index. This is a quantitative, not a relative, measure and permits a better visualization of stand conditions. Furthermore, the use of number of trees as the index avoids confusion with the percentage stocking values discussed previously.

It is deemed desirable, therefore, to limit the use of percentages to percentage stocking values, for comparison of stands of the same species. For interspecies comparisons, the stand-density index expressed in number of trees is desirable, not only because it is a quantitative measure, permitting better visualization of stand conditions, but because it is directly proportional to the percentage relationship (ten times the latter), thus incorporating any advantages of the percentile values, yet avoiding confusion with percentage stocking.

## CONCLUSIONS

The method of determining density of stocking in even-aged stands, which has been described, has the advantages of simplicity, freedom from correlation with age and site index, and general applicability. The equation derived, expressing the relationship between number of trees per acre and average diameter, satisfies the data for 12 of the 14 species investigated and departs but slightly for the thirteenth. Additional species should be investigated for conformity with results presented here. For species, or groups of species, not conforming to this equation, separate reference curves may be established, but it is recommended that the number of trees at an average

<sup>4</sup> For species in which the slope differs from that of the reference curve, a curve paralleling its maximum curve and passing through 1,000 trees at 10.0 inches can be used as a reference curve.

<sup>5</sup> This stand-density index is determined very much as is site index, since it is the number of trees per acre with an average diameter of 10.0 inches which the stand had or will have, assuming that the change in number of trees and average diameter progresses parallel with the reference curve.

diameter of 10 inches be maintained as the basis for stand-density index.

Similar investigations of selection stands will be of value. Schæffer (9) points out that the distribution of trees by diameter in selection stands can also be represented by a straight line on logarithmic graph paper.

#### SUMMARY

The correlation between average diameter of an even-aged stand and the other statistical measures by which the diameter distribution is described permits the use of average diameter alone as a basis for comparing the densities of stands. Of a group of stands of the same average diameter, and, therefore, having the same distribution of diameters, the stand with the greatest number of trees per unit area is obviously the most completely stocked. Age and site quality have no significant effect upon this relationship.

For a given species, the maximum number of trees that it is possible for a stand to have is correlated negatively with the average diameter. The curve representing this relationship assumes a straight-line form when plotted on logarithmic paper and is termed the reference curve.

In 15 groups of data representing 14 species (13 coniferous), the slope of the *number of trees—average diameter* curve was identical for 12 species. The slope for slash pine was slightly greater; that for shortleaf pine was appreciably greater. The heights of these curves, at a given diameter, varied between species.

By means of the reference curve "stand-density index" is derived for a given stand by plotting its number of trees and diameter, passing a line, parallel to the reference curve, through this point, and reading the number of trees per acre at its intersection with the 10-inch ordinate. This expression may be used for comparisons between species and within a species.

Percentage stocking is the percentage expression of the ratio between the number of trees per acre in a given stand and the number, for the same diameter, taken from the maximum curve for the species involved. This expression should be used only for comparisons within a species.

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# YELLOW-RESISTANT LINES OF JERSEY WAKEFIELD CABBAGE<sup>1</sup>

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## INTRODUCTION

In a previous paper<sup>3</sup> the writer has presented the results of a study of the inheritance of resistance to the yellows organism (*Fusarium conglutinans* Wr.) in cultivated and wild cabbage (*Brassica oleracea* L.). In this earlier paper the symptoms of the disease were described, and data were reported on progenies from resistant and susceptible plants of the varieties All Head Early, Copenhagen Market, and Glory of Enkhuizen and from crosses between resistant and susceptible individuals of these three varieties. From the results of this work it was concluded that resistance is controlled by a single dominant gene (*R*) allelomorphic with a single susceptible gene (*r*). It was also pointed out that all plants belonging to the recessive susceptible class (*rr*) did not, under field conditions, become equally affected by the disease. Some died promptly; others, although diseased, continued to live for varying periods and sometimes recovered. It still remains to be determined whether the expression of the disease may be influenced by other factors than the main gene for resistance.

In the course of the investigation referred to, attention was given to the inheritance of resistance in the Jersey Wakefield variety. Resistant individuals of this variety were self-pollinated. The resulting progenies, when tested upon yellows-infested soil, segregated very nearly in the ratio of 3 resistant to 1 susceptible. Moreover, progenies from selfed individuals showed that the resistant class consisted of homozygous plants (*RR*) and heterozygous plants (*Rr*) approximately in the expected ratio of 1 to 2.

The present paper is a report of a continuation of the study of these lines. The investigation had two main objects: (1) To confirm previous findings regarding the homozygous condition for resistance by testing selected lines through succeeding generations and (2) to determine whether it was possible to combine this resistance with the essential features of the Jersey Wakefield variety. The latter problem is important from the practical standpoint, since it has not heretofore been determined whether resistance in cabbage is linked with certain characters that might involve alteration in the standard

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<sup>3</sup> WALKER, J. C. INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE. Jour. Agr. Research 40: 721-745, illus. 1930.

type of a variety. It has, however, been found by past experience that resistant varieties of cabbage are usually somewhat removed in type from the variety from which they were selected.

### METHODS OF EXPERIMENTATION

The methods of seed growing and field testing for resistance previously described<sup>4</sup> were employed in the present investigation. No attempt, however, will be made to classify the various grades of susceptible plants, since the study of this phase of the general problem is reserved for special treatment. All plants showing signs of yellows were permanently marked for the season and were classed as susceptible, and although the degree of infection was recorded it will not be discussed in this paper.

### EXPERIMENTAL RESULTS

As uniform a stock of commercial Jersey Wakefield as could be secured was planted in 1925 on the yellows-infested field plot in Kenosha County, Wis., hereafter referred to as the yellows plot. The plants were distributed among five lots at different locations in the plot. The total number of plants and the percentage that became diseased in each lot are given in Table 1. It will be seen that the amount of disease varied somewhat but that the resistant plants approximated 20 per cent of the total. In all there were 58 plants that remained free from symptoms of disease throughout the season. Some of these were obviously below the standard for the variety and were discarded. About 35 plants were saved and planted in the greenhouse for seed production during the winter of 1925-26.

TABLE 1.—*Yellows in commercial Jersey Wakefield on the field trial plot in 1925*

Lot No.	Total plants	Diseased plants	
	Number	Number	Per cent
1.....	67	59	88.1
2.....	67	58	86.6
3.....	57	44	77.2
4.....	67	54	80.6
5.....	73	58	79.5
Total.....	331	273	82.5

### FIRST-GENERATION SELECTIONS

Owing to losses from decay of the main stems of the plants during the winter, seed was secured from only 14 plants of the group selected from the resistant survivors of Jersey Wakefield. Seed from self-pollination was obtained from 13 plants, and four sib crosses were made. The behavior of the progenies when tested on the yellows plot in 1926 has already been described.<sup>4</sup> Certain of the progenies were retested in 1927, and the results confirmed those previously obtained. It may suffice, therefore, to point out here that segregation was generally that of a monohybrid, and that when all plants showing any sign of disease were placed in the susceptible class this group contained approximately one-fourth of the individuals.

<sup>4</sup> WALKER, J. C. Op. cit.



Whether the progenies selected for resistance to yellows maintained the characteristic appearance of the type was not considered in the earlier paper. At the time of the initial selections only those plants were retained that conformed reasonably closely to the desired type of Jersey Wakefield. It should be remembered, however, that cabbage is an open-pollinated plant and that the individual may therefore be expected to be heterozygous for many of its characters.

Table 2 shows the data on the first-generation progenies. It will be seen that all the progenies segregated for resistance and susceptibility and that approximately 25 per cent of the plants were in the susceptible class. Data on type were taken for the plants in the resistant class, which contained approximately 75 per cent of the total. Since only the outstanding points are considered here, the characteristics ascribed to a given progeny apply to most but not necessarily to all individuals in that progeny. Although this study was not concerned with the inheritance of any character of cabbage other than resistance, in the selection of resistant strains an effort was made to maintain or improve the type.

TABLE 2.—*Results of field trials on yellows-infested soil with progenies of first selections from Jersey Wakefield, as compared with two commercial susceptible varieties*

PROGENIES FROM SELFED PLANTS AND SIB CROSSES OF JERSEY WAKEFIELD

Parent plant No.	Year of trial	Total plants tested		Plants showing yellows	Notes on resistant plants
		Number	Per cent		
3.....	1926	25	16.0		Late maturity; stems too long.
6.....	1926	117	31.6		Late.
7.....	1926	71	25.4		Early; good type.
	1927	22	27.3		Do.
8.....	1926	32	31.3		Early; heads too slender at base.
9.....	1926	85	27.1		Late; heads too slender at base.
10.....	1926	185	14.6		Early; good type.
	1927	92	23.9		Do.
12.....	1926	216	24.1		Late; coarse; irregular.
15.....	1926	94	9.6		Fairly early; good type.
17.....	1926	30	13.3		Late.
19.....	1926	17	29.4		Do.
21.....	1926	180	23.3		Early; good type.
	1927	173	28.3		Do.
29.....	1926	61	19.7		Late; coarse; leafy.
34.....	1926	139	21.6		Late; stems too long.
6X3.....	1926	36	19.4		Poor showing of selfs of two parents.
7X10.....	1926	177	29.4		Early; excellent type.
	1927	180	34.4		Do.
11X12.....	1926	61	32.8		Early; good type.
21X15.....	1926	121	25.6		Early; excellent type.
	1927	31	19.4		Do.
Total.....		2,145	24.6		

COMMERCIAL SUSCEPTIBLE VARIETIES

Variety	Year of trial	Total plants tested	Plants showing yellows
		Number	Per cent
Danish Ballhead.....	1926	41	82.9
	1927	251	96.4
Copenhagen Market.....	1926	240	87.5
	1927	255	80.0

It will be seen that the average date on which many of the progenies matured was later than desirable. Of the strains that were satisfactory for earliness, all but one, that from plant 8, approached the ideal for other type characters. Selections were again made from the best progenies including selfed progenies from plants 7, 10, 15, and 21 and from sib crosses  $7 \times 10$ ,  $11 \times 12$ , and  $21 \times 15$ .

#### SECOND-GENERATION SELECTIONS

From the plants selected from the first-generation progenies, seed was secured in the greenhouse in the winter of 1926-27 by self-pollination, by brother-sister matings, and by crossing with plants of a



FIGURE 1.—Trial plot on which cabbage progenies were tested for resistance to the yellows organism. Bamboo stakes mark diseased plants: A, Self progeny from plant 155, which is segregating into resistant and susceptible classes; B, hybrid progeny  $116 \times 137$  from a cross between homozygous resistant plant 116 and plant 137, which was heterozygous for this character; since the resistant gene is dominant, all plants remained healthy; C, self-progeny from homozygous resistant plant 116; all plants remained healthy; D, susceptible Copenhagen Market variety almost completely killed by the disease. (For further data, see Table 3)

homozygous susceptible line. The breeding behavior of these plants in regard to resistance and susceptibility has been discussed in the earlier paper.<sup>5</sup> It was shown that of the resistant survivors selected from the first-generation progenies approximately one-third were homozygous for resistance ( $RR$ ) and the remainder were heterozygous ( $Rr$ ). The breeding behavior as to type of the progenies from self-pollination and sib crossing is summarized in Table 3. The appearance of certain of the progenies in the field is illustrated in Figure 1.

<sup>5</sup> WALKER, J. C. Op. cit.

TABLE 3.—*Results of field trials on yellows-infested soil with second-generation progenies from Jersey Wakefield, as compared with three commercial susceptible varieties*

PROGENIES FROM SELFED PLANTS AND SIB CROSSES OF JERSEY WAKEFIELD

Parent plant No.	Genotype class of parent plant	Year of trial	Total plants tested	Plants showing yellows	Notes on resistant plants
			Number	Per cent	
116.....	RR	1927	30	0	Shows loss of vigor; late.
117.....	RR	1927	34	0	Early; off-color.
124.....	RR	1927	20	0	Show loss of vigor; late.
135.....	Rr	1927	7	0	Shows loss of vigor.
140.....	RR	1927	41	0	Early; uniform; good type.
		1928	20	0	Do.
		1929	35	0	Do.
154.....	RR	1927	74	0	Do.
		1928	17	0	Do.
		1929	25	0	Do.
115.....	Rr	1927	30	26.7	Late; off-color.
137.....	Rr	1927	26	23.1	Shows loss of vigor; late.
138.....	Rr	1927	13	46.2	Shows loss of vigor.
155.....	Rr	1927	32	34.4	Do.
157.....	Rr	1927	59	28.8	Uneven in maturity.
158.....	Rr	1927	34	26.5	Shows loss of vigor.
124 × 135.....	RR × RR	1927	19	0	Early; good type.
140 × 116.....	RR × RR	1927	36	0	Do.
		1928	30	0	Do.
		1929	53	0	Do.
115 × 135.....	Rr × RR	1927	45	0	Early.
116 × 137.....	RR × Rr	1927	45	0	Late.
117 × 139.....	RR × Rr	1927	30	0	Early.
135 × 115.....	RR × Rr	1927	9	0	
154 × 136.....	RR × Rr	1927	17	0	Do.
158 × 124.....	Rr × RR	1927	24	0	Late.
157 × 117.....	Rr × RR	1927	73	0	Early.
151 × 109.....	Rr × Rr	1927	27	29.6	Late; heads poorly shaped.
138 × 155.....	Rr × Rr	1927	32	25.0	Late.

COMMERCIAL SUSCEPTIBLE VARIETIES

Variety	Year of trial	Total plants tested	Plants showing yellows
		Number	Per cent
Danish Ballhead.....	1927	251	96.4
	1928	298	94.6
	1929	293	91.1
Jersey Wakefield.....	1928	152	90.8
	1927	255	80.0
Copenhagen Market.....	1928	61	90.2

It will be seen that further segregation as to type occurred. Many progenies from self-pollinated plants showed a decided reduction in vigor, as the writer has previously observed in cabbage. Two progenies, however, were outstanding in holding a remarkable degree of uniformity of earliness and other desirable characters along with vigor. These were progenies from the self-pollination of plants 140 and 154. Of the progenies from sib crosses, 124 × 135 and 140 × 116 were equally satisfactory, although of the four parent plants entering into these crosses only plant 140 produced a superior progeny when self-pollinated.

Since the four progenies just mentioned were all homozygous for resistance they became the source of plants for further selection. All other lots were discarded.

SUBSEQUENT GENERATIONS

Since a fairly high degree of uniformity in type and maturity had been attained in the progenies saved from the second generation, no further self-pollination was practiced, except in one case noted later. Groups of plants were planted in isolated places, and cross-pollination by means of insects was allowed to follow. The seeds from plants in each such lot were mixed. The sources of the mother seed plants and the lot numbers of the seed secured were as follows:

Seed lot No.	Source of mother seed plants
20-28-A---	Self progeny of plant 140.
20-201s <sup>6</sup> ----	Progeny 124 × 135.
20-29-C---	Progeny 140 × 116.
20-30-A---	Seed lots 20-28-A and 20-29-C.
20-30-B---	Progenies 140 × 116, 201s, and seed lot 20-29-C.

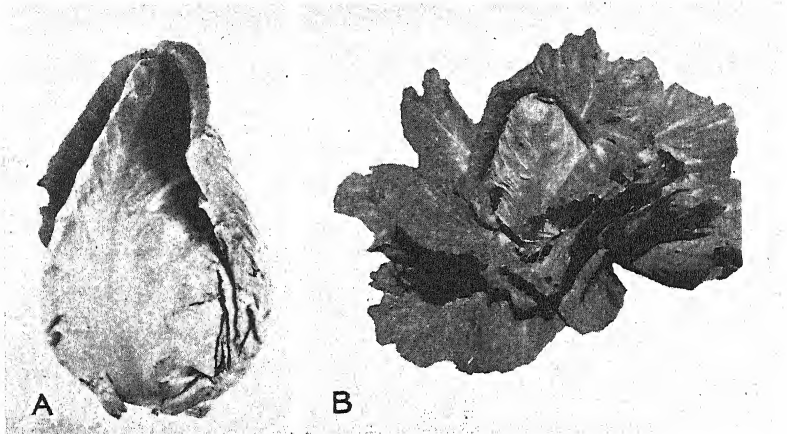


FIGURE 2.—Sample individuals taken from homozygous resistant lines of Jersey Wakefield cabbage, at maturity. These are reasonably close to the types represented in acceptable commercial susceptible stocks of this variety. The conical-shaped head is characteristic. Note that the outer leaves of the head extend to the apex or overlap it slightly. A, Head taken from seed lot 20-29-C. B, Plant from lot 20-201s

All the seed lots listed were tested on yellows-infested soil either in the yellows plot or in the greenhouse. The results of these trials (Table 4) show that the plants were as completely resistant as the progenies from which they were derived. It may be concluded therefore, that resistance was completely fixed in these lines. Sample individuals are illustrated in Figure 2.

TABLE 4.—Results of tests on yellows-infested soil of strains derived from homozygous resistant second-generation progenies

Seed lot No.	Where tested	Plants tested	Plants diseased
		Number	Number
20-28-A-----	Field-----	280	None.
20-201s-----	do-----	170	Do.
20-29-C-----	do-----	365	Do.
20-30-A-----	Greenhouse-----	94	Do.
20-30-B-----	do-----	72	Do.
	Field-----	300	Do.

<sup>6</sup> In this lot only one plant blossomed. The blooming plant was selfed.

## COMPARISON OF THE RESISTANT STRAINS WITH COMMERCIAL JERSEY WAKEFIELD

It has been pointed out that during the course of this work a special effort was made to select for uniformity of maturity and for the desirable characters of the Jersey Wakefield variety. After seed had been secured in sufficient quantity, comparative tests were made in 1931 at Madison, Wis., of three of the lots listed on page 644, together with four lots of Jersey Wakefield secured from commercial sources. Each lot was planted in double rows, 30 plants to a row, on soil free from yellows, and the complete series was run in triplicate. Seed was sown in flats in the greenhouse on April 1. Plants were pricked out into flats and placed in a coldframe on April 17 and set into the field on May 22. The season was decidedly favorable for cabbage development, except that at the time when heading began the air temperature was unusually high. This appeared to increase somewhat the degree of irregularity in maturity.

The first mature heads were noted on the fifty-third day after transplanting. The number of mature marketable heads was recorded at 3-day to 4-day intervals thereafter. Table 5 shows the percentage of mature plants in each lot on the fifty-third day and at regular intervals up to the seventy-fifth day. It will be seen that considerable variation occurred among the four commercial lots. Lot 4 had the largest number of mature heads on the fifty-third day, and this lot continued to mature more rapidly than the others. Lot 2 was decidedly the slowest of the commercial lots in maturing.

TABLE 5.—*Number of days from transplanting to maturity for various commercial and resistant strains of Jersey Wakefield at Madison, Wis., 1931*

Period from transplanting to maturity (days)	Percentage of plants reaching mature-head stage in—						
	Commercial strain No.				Resistant strain No.		
	1	2	3	4	20-28-A	20-30-A	20-30-B
53.....	16	8	13	24	37	7	8
56.....	29	13	23	33	63	17	23
59.....	47	23	38	46	73	25	34
62.....	55	28	50	59	81	36	51
65.....	78	58	67	80	90	56	76
68.....	86	73	80	90	92	68	86
72.....	92	79	86	92	95	72	91
75.....	93	85	90	94	97	80	94

Resistant strain 20-28-A was distinctly superior to all other lots not only in the number mature on the fifty-third day but also in the promptness with which the remaining plants came to head. It is to be recalled that this lot was derived from the selfed progeny of plant 140. Each of the two other resistant strains was derived from plants from several progenies. They did not mature as promptly as 20-28-A. 20-30-B was better in this respect than 20-30-A. Both were in the range represented in the commercial lots. These data show that so far as time of maturity is concerned the resistant strains are within the limits represented by the commercial variety from which they were originally derived.

For comparing the relative earliness of the various susceptible and resistant lots, the most satisfactory criterion is the time required for 50 per cent of the plants in a given lot to reach maturity. On this basis 20-28-A was distinctly superior to all other lots, for 63 per cent of the plants of this lot were mature 56 days after transplanting. No other lot reached this stage until the sixty-second day, when susceptible lots 1, 3, and 4 and resistant lot 20-30-B showed 50 per cent or more mature plants. Susceptible lot 2 and resistant lot 20-30-A did not reach this stage of development until the sixty-fifth day.

To secure a comparison of other characters, certain measurements were made of the first 50 heads that matured in each lot. These measurements are given in Table 6. The three resistant strains were uniformly about 2 cm less in height of plant than the susceptible lots. Susceptible lot 2 had the greatest spread of plant; all other lots were reasonably similar in this respect. In number of outer leaves 20-28-A was lowest, and susceptible lot 2 was highest. The other five lots showed little variation in this character.

In weight of outer leaves there was considerable variation. This is an important character and should be considered in relation to weight of head. Seasonal conditions, of course, influence greatly the proportion of outer leaf to head. In 1931 at Madison the proportion of weight of leaf to weight of head was unusually high, probably because of the extremely high temperatures when the plants were approaching maturity. It will thus be seen in the calculated ratios of leaf weight to head weight that the latter ranged from 50 to 91 per cent of the former. Under most favorable seasonal conditions it might have averaged 150 to 200 per cent. Under the conditions of this experiment susceptible lot 2 showed the largest amount of leaf weight in proportion to head weight. Resistant strain 20-30-A was also relatively high in leaf weight. Lots 3 and 4 were the lowest of the susceptible strains in relative leafiness, and resistant strain 20-30-B was very close to them. Of all the strains tested, however, 20-28-A had the lowest leaf weight in proportion to head weight.

The various measurements of length and diameter of stem show no important differences among the several strains. The length of head from top to base and the diameter (taken at the point of greatest diameter) were considered in relation to each other. The length ranged from 1.39 times the diameter in commercial strain 2 to 1.64 times the diameter in resistant strain 20-30-B. Length of core is also an important feature and may be considered in relation to length of head. Relative shortness of core is, of course, desirable. Ratio of length of core to length of head ranged from 1:2.08 in resistant strain 20-28-A to 1:2.57 in 20-30-B. Resistant strain 20-30-A and all the susceptible lots fell between these extremes.

The characters of outer leaves in the various lots showed no tangible differences except in color and bloom. In the resistant strains there was slightly less bloom than in the susceptible ones. Reduction in bloom tended to give a yellower cast to the foliage as well as to the outer leaves of the head. This deviation, however, was not considered sufficient to detract materially from the commercial value of the resistant strains. Furthermore it is to be noted that the amount of bloom varies with season and soil.

TABLE 6.—Physical measurements of various commercial and resistant strains of Jersey Wakefield, grown at Madison, Wis., 1931

Measurements	Commercial strain No.—				Resistant strain No.—		
	1	2	3	4	20-28-A	20-30-A	20-30-B
Height of plant.....centimeters.....	23.28±0.34	24.5±0.42	23.54±0.42	23.10±0.23	21.28±0.20	21.16±0.32	20.88±0.25
Spread of plant.....do.....	48.76±.51	50.90±.67	46.56±.55	48.0±.64	46.06±.45	47.1±.75	46.8±.40
Outer leaves.....number.....	13.8±.37	15.14±.32	13.5±.38	13.9±.32	12.96±.34	14.0±.32	14.0±.23
Weight of outer leaves.....grams.....	398.5±12.37	534.9±21.40	363.1±11.85	413.8±10.18	359.1±8.03	402.0±13.25	410.5±8.49
Length of stem:							
From soil to base of first leaf.....centimeters.....	4.54±.20	3.87±.16	3.17±.13	3.57±.16	2.95±.10	3.31±.18	2.68±.12
From base of first leaf to base of head.....do.....	5.89±.13	5.10±.11	5.32±.10	5.52±.14	5.34±.10	5.39±.11	5.76±.10
Diameter of stem:							
At soil line.....do.....	1.46±.02	1.08±.02	1.30±.02	1.54±.02	1.46±.02	1.38±.02	1.30±.03
At first leaf.....do.....	2.22±.04	2.91±.06	2.45±.04	2.35±.03	2.76±.03	2.80±.04	2.40±.03
At base of head.....do.....	3.08±.04	3.63±.04	3.45±.03	3.38±.03	3.56±.03	3.57±.03	3.63±.06
Weight of head.....grams.....	259.4±8.41	304.2±12.57	262.6±8.70	290.0±9.31	329.7±7.70	239.4±8.58	295.2±9.58
Ratio of weight of outer leaves to weight of head.....do.....	1.0±.65	1.0±.57	1.0±.72	1.0±.70	1.0±.92	1.0±.60	1.0±.72
Length of head.....centimeters.....	11.05±.16	11.04±.18	11.44±.18	11.53±.17	11.46±.17	10.45±.14	12.6±.15
Diameter of head.....do.....	7.12±.10	7.97±.15	7.05±.11	7.49±.09	8.20±.10	7.06±.11	7.06±.11
Diameter of core.....do.....	4.75±.10	4.47±.11	4.97±.08	5.28±.10	5.30±.11	4.63±.07	4.59±.09
Diameter of core <sup>a</sup> .....do.....	2.56±.02	2.77±.03	2.60±.03	2.79±.03	2.84±.03	2.72±.03	2.79±.03
Ratio of diameter of head to length of head.....do.....	1.0±1.55	1.0±1.39	1.0±1.62	1.0±1.54	1.0±1.40	1.0±1.48	1.0±1.64
Ratio of length of core to length of head.....do.....	1.0±2.33	1.0±2.47	1.0±2.30	1.0±2.20	1.0±2.08	1.0±2.26	1.0±2.37

<sup>a</sup> Taken at point of maximum diameter.<sup>b</sup> Taken midway between base and apex.

## DISCUSSION

In the course of this report it has been shown that homozygous resistant progenies selected from commercial Jersey Wakefield have been repeatedly secured. Rigid selection for conformity to type within these lines was practiced and three distinct strains were developed and carefully compared for maturity and important physical measurements with four commercial stocks of the original variety. One of these resistant lines was derived directly from a selfed progeny and each of the two other lines from a combination of two or more resistant progenies that were allowed to cross-pollinate. The object in varying the procedure was to determine the feasibility of practicing self-pollination until resistance was fixed and other desirable characters were also more stable and then continuing the line by sib crossing within the progeny. Although some progenies showed distinct loss of vigor from selfing, others showed no detrimental effects. One of the latter (the self progeny from plant 140) was sib crossed and yielded seed lot 20-28-A.

The data in Tables 5 and 6 show that two of the resistant lines, 20-28-A and 20-30-B, were reasonably like the better commercial stocks 1, 3, and 4. Commercial Jersey Wakefield 2 was obviously a poor stock of the variety, since it was later in maturity than is desirable and tended to show excessive leafiness. Of the two resistant strains, 20-28-A was distinctly superior to all strains in earliness and in proportion of leaf weight to head weight. It had, however, two objectionable features, namely, the diameter of the head, which was relatively greater, and the core, which tended to be longer, than those of acceptable commercial strains. Resistant strain 20-30-B, on the other hand, was much closer to the three best susceptible stocks. Although it was not so early as 20-28-A, it compared favorably in this respect with susceptible stocks 1, 3, and 4. Furthermore, it was nearer the accepted commercial strains in head diameter and core length. In view of these characters, it is probably better fitted for increase and general use than is 20-28-A.

As a result of this investigation, the writer wishes to emphasize the desirability of most careful and rigid selection for type in the course of improvement of cabbage for resistance to yellows. In the work presented here it has been possible to combine resistance with the more important type characters found in acceptable commercial stocks, without prolonging the program through repeated back crossing to the susceptible stock. The latter procedure should be used, however, when it becomes necessary to accumulate in the resistant line the genes controlling desirable characters.

## SUMMARY

The evidence presented in this paper confirms the results reported earlier, from which it was concluded that a single-factor difference exists between plants highly resistant or highly susceptible to the yellows organism. It is further demonstrated that from resistant survivors occurring when the Jersey Wakefield cabbage is planted on infested soil homozygous resistant pure lines can be secured which continue to be completely resistant in succeeding generations.

In the course of this investigation it was found that by rigid selection for type and uniformity of maturity it is possible to combine the desirable characters of this variety with resistance.



# THE EFFECTS OF NONBENEFICIAL NODULE BACTERIA ON AUSTRIAN WINTER PEA<sup>1</sup>

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## INTRODUCTION

When typical nodules form on the roots of legumes the bacteria which they contain are presumed to be working in harmony with the plants, furnishing nitrogenous compounds manufactured from the gaseous nitrogen of the air. Apparently this is not always the case, however, for occasionally a condition arises in which there is no doubt that the nodule organisms have exerted a detrimental effect. In many other instances it is probable that the bacteria live on the roots of the legume without appreciably influencing the plant. It is to be expected that all grades of efficiency exist among the legume bacteria.

## REVIEW OF LITERATURE

The nature of the association of legume and nodule bacteria has long been in doubt. By some it has been regarded as parasitism, but if it is so, it can only be considered as theoretical parasitism. Voorhees and Lipman (12, p. 92)<sup>3</sup> state:

\* \* \* in the very definition of the term, parasitism involves a more or less marked injury to the host, which, at best, derives no benefit from the invading organism. In the phenomenon under consideration, however, there is, under normal conditions, a distinct gain to the host plant, and hence the term "parasitism" is not applicable.

Woronine (13), who first discovered bacteria in nodules, referred to them as parasites. Smith (10, v. 2, p. 97), considered nodule bacteria as parasites, since they pass through the same stages as disease-producing organisms. Jones (7) attributes parasitic tendencies to mung-bean nodules growing under submerged conditions.

Eaton (4) has reported that nodule production on soybeans bears a direct relation to light, and Leonard (8), also working with soybeans, has shown that a lack of light, or interference with the organs utilizing light, reduces the beneficial effects of symbiosis in accordance with the severity of the light deficiency. Thornton (11) states that parasitism in nodule bacteria is induced by cutting off the carbohydrate supply.

The appearance of ill effects among legume plants growing under optimum conditions is due to another type of parasitism—a parasitism inherent in the invading organism. In cases of this kind the organism enters the roots and forms nodules, but these are not beneficial.

<sup>1</sup> Received for publication June 8, 1932; issued May, 1933.

<sup>2</sup> The services of Daniel Ready, Bureau of Chemistry and Soils, and of A. P. Kerr, Louisiana Agricultural Experiment Station, in making nitrogen and other analyses, and of J. B. Thompson, Bureau of Plant Industry, in assisting in field and laboratory work, are greatly appreciated.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 663.

Hiltner (6) calls attention to parasitic nodules on locust and differentiates between these and beneficial nodules by their location on the roots. The former were distributed all over the root, whereas the latter were located at the crown.

Helz, Baldwin, and Fred (5) citing 16 papers on this subject, reported a wide variation in the effects produced by strains of nodule bacteria of the vetch and pea group.

Briscoe<sup>4</sup> reported greenhouse work in which poor and good strains were used in combination, and the mixture produced as satisfactory results as the good strain alone. A means of correcting the unadaptability of strains is reported by Wunchik (14), who has indicated that by plant passage an unadapted strain may become better adapted to a strange host, and Allen and Baldwin (1) have confirmed this finding. That the organisms in nodules on the same plant may consist of both good and poor strains has been shown by Dunham and Baldwin (3).

#### EXPERIMENTAL MATERIALS AND OUTLINE OF WORK

In 1929 observations were made at Jeanerette, Iberia Parish, La., on a field of Austrian winter peas (*Pisum arvense* L.) which had been planted without artificial inoculation on the low-lying black neutral alluvial soil of that locality. After a month or so of growth it was definitely apparent that the crop would be a failure; the field contained mainly yellow stunted plants with an occasional vigorous individual. Samples of the plants were collected and sent to the senior author at Washington, D. C. The roots of most of the plants examined, whether normal or stunted, were apparently healthy, and all the plants had nodules on their roots. It was noticed, however, that the nodules on the stunted plants were small, globular in shape, and rather evenly distributed over the roots, whereas those on the vigorous plants tended to be convolute or branched and were largely confined to the crown roots. Cultures were isolated from nodules of both types of plants and tested on Austrian winter pea. These cultures produced nodules on the pea under controlled conditions and, in addition, gave rise to effects similar to those noticed on the plants from which they came. In other words, the bacteria from the stunted-plant nodules produced what might be called parasitic effects, whereas those from vigorous plants produced good plants. When these preliminary tests were completed cultures of the proper nodule bacteria were prepared on agar in test tubes and sent to Jeanerette, La. (Table 1.) There they were applied to seed by adding water to the tube, shaking thoroughly to secure a suspension of the organisms, and moistening the seed with the mixture. The seeds were sown promptly after treatment at the rate of 1 pound per each 10 by 60 foot plot. To prevent transfer of the cultures, vessels and hands were thoroughly rinsed in running tap water between each treatment. No disinfectants were used. Before the seeds were sown basic slag at the rate of 600 pounds per acre and ground oyster shells at the rate of 3,600 pounds per acre were applied each to one-third of the plots in Austrian winter pea; the remaining third were left untreated. Figure 1 shows the arrangement of the plots.

<sup>4</sup>BRISCOE, C. F. INOCULATION OF SOYBEANS. Miss. Agr. Expt. Sta. Press Circ. 419, 3 p. 1932. [Mimeographed.]

TABLE 1.—Source of the nodule bacteria cultures used in the experiments

Stock No.	Diagram No. (fig. 1)	Host plant from which bacteria were isolated	Source of cultures
473 <sup>a</sup> .....	1	Alaska garden pea.....	W. H. Wright, University of Wisconsin; his No. P3. 1927.
484 <sup>a</sup> .....	7	do.....	I. L. Baldwin, University of Wisconsin; his No. 15 or 310 1927.
510.....	3	Austrian winter pea.....	Jeanerette, La. 1929.
511.....	6	do.....	Do.
512.....	4	do.....	Do.
513 <sup>b</sup> .....	2	do.....	Do.
515 <sup>b</sup> .....	8	do.....	Do.
518 <sup>c</sup> .....	5	do.....	Rossllyn, Va. 1930.
None <sup>d</sup> .....			
548.....		Lotissiana vetch.....	Jeanerette, La. 1931.
549.....		do.....	Do.
549.....		do.....	Do.
550.....		do.....	Do.

<sup>a</sup> The complete history of this culture is not available, but judging from the response obtained with Austrian winter peas, it is believed to be a low nitrogen-fixing strain.

<sup>b</sup> This culture was isolated from plants that showed no benefit from the presence of nodule organisms.

<sup>c</sup> This culture was isolated from a slightly pink nodule apparently produced by a nonbeneficial culture on plants showing symptoms of being adversely affected by nodule bacteria. From subsequent results it is presumed that this culture either changed its character rapidly, by means of one plant passage, or that through some unknown means an efficient organism was harbored in the nodule from which it came.

<sup>d</sup> Designation used for untreated areas.

2 RIDGES UNINOCULATED AUSTRIAN WINTER PEAS

NORTH

G.P.	5	6	0	8	7	0
F.P.	2	0	3	4	0	1
A.P.	0	7	8	0	5	6
H.V.	4	3	0	1	2	0
G.P.	8	0	5	6	0	7
F.P.	0	1	2	0	3	4

A.P.	5	6	0	8	7	0
H.V.	2	0	3	4	0	1
G.P.	0	7	8	0	5	6
F.P.	4	3	0	1	2	0
A.P.	8	0	5	6	0	7
H.V.	0	1	2	0	3	4

A

NORTH

SECOND PLANTING

CHECK	5	6	0	7	8	0
B.S.	5	6	0	7	8	0
L.	5	6	0	7	8	0
CHECK	2	0	4	3	0	1
B.S.	2	0	4	3	0	1
L.	2	0	4	3	0	1

FIRST PLANTING

CHECK	0	7	8	0	5	6
B.S.	0	7	8	0	5	6
L.	0	7	8	0	5	6
CHECK	4	3	0	1	2	0
B.S.	4	3	0	1	2	0
L.	4	3	0	1	2	0
CHECK	8	0	5	6	0	7
B.S.	8	0	5	6	0	7
L.	8	0	5	6	0	7
CHECK	0	1	2	0	3	4
B.S.	0	1	2	0	3	4
L.	0	1	2	0	3	4

B

The mixed-legume plots and one of the Austrian winter-pea plots (fig. 1, B) were seeded October 22, 1930; the second sowing of Austrian winter peas, a duplicate of the first, was made November 3, 1930. The plots seeded to mixed legumes were 10 by 60 feet, and those seeded to Austrian winter peas were 10 by 20 feet. Samples of the black silt soil taken from seven representative spots in the mixed-legume planting gave a pH value of 6.75, indicating a uniform hydrogen-ion concentration. Six composites of individual samples from three different spots in the first planting of the Austrian winter peas were more variable in reaction, the pH values ranging from 6.57 to 7.88. Obviously there was little need for a neutralizing agent in this soil.

## FIELD OBSERVATIONS

### GROWTH AND NODULATION

On January 31, 1931, plants grown from seed treated with cultures 510, 511, and 518 were observed to be greener than those in other plots of the first planting of Austrian winter peas, in some of which the plants were turning yellow and a few were dying. On the plots containing mostly poorly colored plants, an occasional rather vigorous plant was noticed. All plants, even those in the worst condition, had nodules on their roots. As in earlier studies (9) it was found that on the better plants the nodules tended to branch or become convolute, whereas on most of the poor plants the nodules were smaller and globular in shape, the former type being placed rather generally around the crown, and the latter scattered more or less evenly over the roots.

On February 17, 1931, a second examination of representative plants was made. This revealed much the same condition as the first. (Table 2.) At this time there was no evidence that either the basic slag or the lime had influenced the growth of the plants or the formation of nodules.

TABLE 2.—*Growth and nodulation of Austrian winter peas and other legumes at Jeanerette, La., February 17, 1931*

Name of plant <sup>a</sup>	Inoculation treatment	Growth	Nodulation
Canada field pea.....	510	Excellent.....	Normal. <sup>b</sup>
Alaska garden pea.....	None	Fair.....	Scattered.
Hairy vetch.....	None	Poor.....	Plentiful.
Austrian winter pea (2).....	512	Excellent.....	Normal.
Austrian winter pea.....	484	Poor.....	Scarce.
Do.....	484	Fair.....	Do.
Do.....	518	Poor.....	Do.
Austrian winter pea (2).....	518	Excellent.....	Normal.
Austrian winter pea (3).....	511	do.....	Do.
Austrian winter pea (6).....	None	Poor.....	Scarce.
Austrian winter pea (2).....	510	Excellent.....	Normal.
Austrian winter pea (3).....	513	Poor.....	Scarce.

<sup>a</sup> Numbers in parentheses indicate the number of samples examined.

<sup>b</sup> Normal means tendency to branch and locate near the crown, all other notations in this column refer to scattered globular nodules.

Plants of Austrian winter pea from seed inoculated with culture 510 accidentally mixed with the seed of hairy vetch were very good,

and plants from the latter were much better than other vetch plants from untreated seed, most of which did not exceed 4 inches in height. All vetch plants had nodules. Whether the beneficial effect of the association of vetch with Austrian winter pea was due to a transference of adapted organisms or to the utilization of by-products is not known, but it is an interesting lead to consider. The seed of Canada field peas and Alaska garden peas did not germinate satisfactorily, and the crop that was made was not satisfactory.

A better picture of the differences between excellent and poor growth and between the two types of nodulation is shown in Figure 2. Although some of the roots were broken off in removing the gummy soil and many nodules are believed to have been lost in the washing process, Figure 2 adequately illustrates the extreme variation in root system and type of nodulation resulting from treatments with beneficial and nonbeneficial cultures. No differences were observed between the untreated plants and roots and those treated with nonbeneficial cultures.

The Austrian-winter-pea plants from the ridged beds were better than those from the flat bed, but not nearly so good as plants from the areas inoculated with good cultures. Possibly the ridging limited the water supply and thereby interfered with the activities of the native nonbeneficial nodule bacteria.

During the course of the experiment, more especially in the later stages, some of the poor plants which survived the earlier effects of treatment took on renewed life. This is presumed to have been due to the spreading of good nodule bacteria by means of dust, implements, insects, man, or beast. It is reasonable to expect, and experience offers a slight basis for believing, that infections may occur with bacteria of good, bad, and intermediate efficiency, so it is quite possible that where there is an apparent turn for the better, the more efficient organisms may have obtained the upper hand or the progeny of the nonbeneficial organisms may have become better adapted to the plant while within the nodule.

#### CROP YIELDS

By harvest time a great many of the vigorous growing Austrian-winter-pea plants had lost a rather large proportion of their lower leaves by shedding or decay. Plant lice may have contributed to the losses, since they were plentiful on the plants of the early sowing. The pea vines, which had grown over the dividing furrows, were thrown back on their respective plots before harvesting. The vines were cut with a scythe during rather dry weather and weighed promptly after cutting.

The larger weeds were separated from the cut material, but it was not practical to remove all the little weeds and grass that had grown up in the plots where pea plants had died. It is to be expected that the weight of the grass and weeds will partly compensate for the dead pea plants and may therefore give a false picture in the tabulation of yield data. Where the growth of the winter peas was good and the vines thoroughly covered the ground, weeds and grass were largely suppressed.

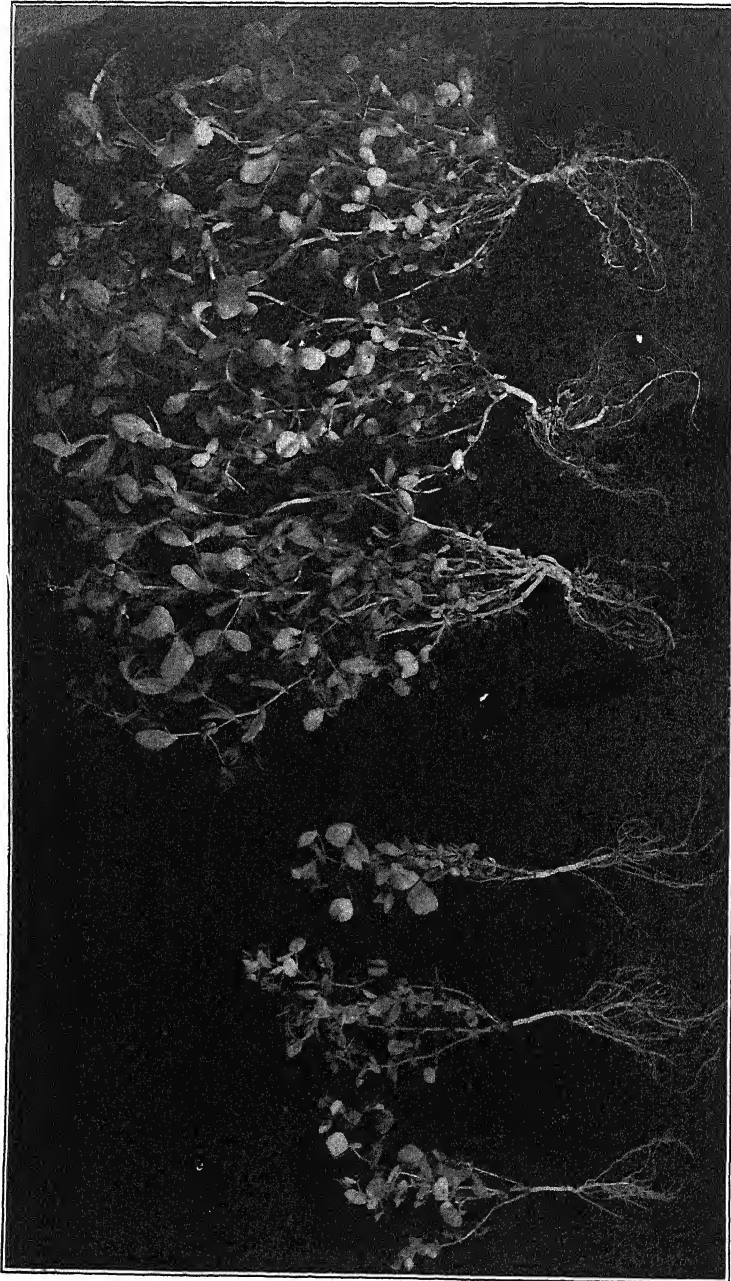


FIGURE 2.—Representative Austrian winter-pea plants from plots treated with a beneficial nodule culture (right), and with a nonbeneficial nodule culture (left)

TABLE 3.—Yields of Austrian winter peas from plots inoculated with a beneficial culture and from untreated plots

[Planted October 22, 1930, harvested April 24, 1931]

Inoculation treatment	Yield of hay from 10 by 20 foot plot		Moisture in green pea vines	Inoculation treatment	Yield of hay from 10 by 20 foot plot		Moisture in green pea vines
	Green	Moisture free			Green	Moisture free	
	<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>
511.....	81	13. 98	82. 74	None.....	6	1. 05	82. 56
None.....	21	4. 35	79. 28	511.....	79	19. 97	74. 72
511.....	86	26. 55	69. 13	None.....	16	3. 35	79. 04

Table 3 shows the yields from uninoculated Austrian winter peas compared with those from peas inoculated with a beneficial culture. The data indicate an increase of 485 per cent in the yield from the inoculated plots as compared with that from the uninoculated. If such improvement can be made fairly consistently in these soils, it is apparent that the addition of the proper organisms will mean the difference between success and failure.

A comparison of the effects of basic slag and lime on Austrian winter peas receiving different cultures is given in Table 4. The data shown in Table 4 indicate no decided superiority in crop yield as a result of the application of basic slag or lime.

TABLE 4.—Plot yields in pounds (green weight) of Austrian winter-pea hay per 10 by 20 foot plots, as affected by inoculation, basic slag, or lime

[Planted October 22, 1930; harvested, except as otherwise noted, May 11, 1931]

Inoculation treatment	Yield with—			Total yield
	Lime	Basic slag	No addition	
512.....	80	92	75	247
510.....	73	86	71	230
None.....	61	61	50	172
513.....	44	55	66	165
473.....	43	54	45	142
None.....	29	33	53	115
Total.....	330	381	360	-----
484.....	31	45	60	136
None.....	35	38	44	117
511.....	62	72	57	191
518.....	55	76	63	194
None.....	42	45	47	134
515.....	46	59	55	160
Total.....	271	335	326	-----
None.....	62	59	51	172
513.....	47	52	41	140
473.....	50	45	36	131
None.....	56	52	44	152
510.....	47	59	48	154
512.....	53	62	58	173
Total.....	315	329	278	-----

TABLE 4.—*Plot yields in pounds (green weight) of Austrian winter-pea hay per 10 by 20 foot plots, as affected by inoculation, basic slag, or lime—Continued*

Inoculation treatment	Yield with—			Total yield
	Lime	Basic Slag	No addition	
511.....	56	59	63	178
518.....	64	51	51	166
None.....	50	50	36	136
515.....	47	43	30	120
484.....	28	29	16	73
None.....	43	45	27	115
Total.....	288	277	223	
473.....	47	48	44	139
None.....	44	45	43	132
510.....	50	55	43	148
512.....	51	55	49	155
None.....	19	18	20	57
513.....	31	31	30	92
Total.....	242	252	229	
None.....	62	59	63	184
515.....	42	44	46	132
484.....	37	45	42	124
None.....	16	6	21	43
511 <sup>a</sup> .....	79	86	81	246
518 <sup>a</sup> .....	42	47	37	126
Total.....	278	287	290	
Grand total.....	1,724	1,861	1,706	

<sup>a</sup> Harvested Apr. 24, 1931.

Table 5 shows the relative value of the different inoculation cultures.

TABLE 5.—*Summary of the hay-crop yields of Austrian winter peas from planting made October 22, 1930, and harvested May 11, 1931*

Inoculation treatment	Plots		Average yield of dry hay	Inoculation treatment	Plots		Average yield of dry hay
	Number	Pounds			Number	Pounds	
None.....	36	42.4	518.....	9	54.0		
473.....	9	45.8	511.....	6	61.5		
513.....	9	44.1	484.....	9	37.0		
510.....	9	59.1	515.....	9	47.3		
512.....	9	63.9					

Even with the larger proportion of weeds and grass in the areas inoculated with nonbeneficial bacteria, there is apparently an appreciable increment in crop yield due to cultures 510, 512, 518, and 511, whereas cultures 473, 513, 484, and 515 are not greatly superior to no treatment.

The second planting of Austrian winter peas was substantially the same in layout as the first, yet the results were not the same. The whole crop, regardless of treatment, gave no significant differences attributable to basic slag, lime, or inoculation. (Table 6.) The total yield of dry hay from the 36 plots was as follows: 1,624 pounds from lime, 1,680 pounds from basic slag, and 1,616 pounds from no treat-



ment. The lack of variation in yields from the second planting may have been due to a scattering of good types of nodule organisms by an inundation.

TABLE 6.—Average yields of dry hay from plots of the second planting of Austrian winter peas, sown November 3, 1930, and harvested May 22, 1931

Inoculation treatment	Plots	Yield of dry hay		Inoculation treatment	Plots	Yield of dry hay	
		Total	Average per plot			Total	Average per plot
	Number	Pounds	Pounds		Number	Pounds	Pounds
None.....	36	1,680	46.7	511.....	9	444	49.3
473.....	9	367	40.8	484.....	9	400	44.4
513.....	9	387	43.0	515.....	9	411	45.7
510.....	9	396	44.0	Total or mean..	108	4,920	45.5
512.....	9	382	42.4				
518.....	9	453	50.3				

#### NITROGEN IN AUSTRIAN WINTER PEA AS AFFECTED BY INOCULATION

Samples of pure winter-pea hay from a few plots were harvested February 16, 1931, dried thoroughly, and ground for analysis. The results are given in Table 7.

Comment on these results is almost unnecessary since they are of the same general nature as the other evidence given of the effect of nodule organisms on Austrian winter peas.

TABLE 7.—Nitrogen analyses of field-grown Austrian winter-pea hay harvested at Jeanerette, La., February 16, 1931

[Planted October 22, 1930]

Inoculation treatment	10 plants (dry weight)	Nitrogen	Total nitrogen
	Grams	Per cent	Grams
None.....	5.3	0.94	0.05
511.....	37.25	3.40	1.27
513.....	13.46	.86	.12
518.....	40.77	3.08	1.25

#### GREENHOUSE EXPERIMENTS

In a series of greenhouse experiments at Rosslyn, Va., Austrian winter peas were grown in sterilized sand moistened with Bryan-Crone's nutrient solution (2). Before the seed was sown it was sterilized with 1:500 mercuric chloride, washed thoroughly with sterile tap water, and inoculated with bacteria from test-tube cultures, the usual precautions being taken to prevent the transfer of nodule bacteria from one series to another. Each treatment was replicated 10 times and each replication usually consisted of about five plants. The data are shown in Table 8.

TABLE 8.—Nitrogen fixation in greenhouse plantings of Austrian winter peas

Inoculation treatment	Duration of experiment	Nodules per plant	Dry weight (50 plants)	Nitrogen	Total nitrogen
		Average number	Grams	Per cent	Gram
None.....	Oct. 28, 1929 to Dec. 12, 1929 (45 days)	3	13.00	2.60	0.339
473.....		15	12.50	3.33	.416
484.....		16	12.50	2.38	.300
510.....		15	15.50	3.04	.471
511.....		23	23.00	2.94	.675
512.....		21	21.50	3.08	.664
513.....		21	21.50	2.15	.461
515.....		23	23.00	2.40	.550
518.....		20	20.50	3.09	.632
None.....		3	15.60	1.30	.203
510.....	Feb. 14, 1931 to Apr. 8, 1931 (53 days)	15	18.50	2.86	.532
511.....		16	20.75	2.74	.568
513.....		96	15.90	1.22	.194
518.....	Apr. 4, 1931 to May 25, 1931 (51 days)	13	26.85	2.36	.635
None.....		1	12.80	1.72	.220
512.....		15	13.80	2.70	.372
513.....		135	13.20	2.04	.269
548.....		90	11.40	1.66	.189
549.....		99	12.20	1.68	.205
550.....		112	11.60	1.66	.192
512+513.....		29	11.70	1.94	.227
513+NaNO <sub>3</sub> .....		44	14.90	2.12	.316

In the first experiment, culture 510 apparently gave poor results, so far as dry weight and total nitrogen are concerned, yet the percentage of nitrogen rather closely parallels that of the other beneficial cultures. Considering matters on the basis of total nitrogen apparently fixed by nodule organisms, it will be noted that the plants from only one culture (484) contained less nitrogen than the control. Apparently the so-called nonbeneficial cultures were slightly beneficial when the quantity of nitrogen fixed is considered. However, in the second experiment the nitrogen fixed by the nonbeneficial culture (513) was actually less than that of the control and much less than that of any of the three beneficial cultures employed, and in the last experiment this nonbeneficial culture was slightly better than the control. When the two types of organisms (512 and 513) were used together the total nitrogen fixed was not greater than that fixed by the nonbeneficial organism alone. However, the addition of 0.1 per cent sodium nitrate to the solution originally used to moisten the sand (subsequent waterings being made without nitrogen additions) helped the plants inoculated with a nonbeneficial organism considerably.

#### SOURCE OF THE NONBENEFICIAL ORGANISMS

The fact is evident that nodule bacteria beneficial and detrimental to Austrian winter peas exist in some of the soils around Jeanerette, La., the nonbeneficial ones evidently predominating. In seeking an explanation for the presence of these organisms it was found that plants of Louisiana vetch (*Vicia ludoviciana* Nutt.) grew plentifully and luxuriantly around the fields and in waste places. The roots of these wild plants are well populated with nodules. It is therefore reasonable to believe that the organisms causing these nodules are abundantly present in the soil. It is customary to consider that the organisms of the common vetch will cross-inoculate with garden

peas, field peas, Austrian winter peas, and other legumes, although data concerning the group relations of Louisiana vetch have not been worked out. However, acting on the supposition that the bacteria of this legume are related to those of the Austrian winter pea but not satisfactorily adapted to the latter, the writers made isolations from nodules for comparison with the nonbeneficial bacteria obtained from Austrian winter peas.

After the cultures from Louisiana vetch were purified they were applied to Austrian winter pea and Louisiana vetch seed in the greenhouse under controlled conditions, as indicated in Table 8. These cultures produced nodules on their specific hosts. The results with Austrian winter peas indicate that these Louisiana vetch nodule organisms produce the same effect as those of the nonbeneficial type whose effects have been previously noted in this paper. From April 4 to May 25, 1931, the Austrian winter-pea plants grown from seed treated with three cultures of nodule organisms of Louisiana vetch had much the same appearance as those from seed treated with a nonbeneficial culture isolated from Austrian winter pea nodules. (Fig. 3.) As compared with the control, Louisiana vetch organisms (548, 549, and 550) contributed no nitrogen to the Austrian winter peas; in fact they caused a slight loss. (Table 8.)

Nodules produced by Louisiana vetch cultures were plentiful, scattered, and globular. Nodules of this type are shown in Figures 4 and 5 in comparison with nodules produced by beneficial and non-beneficial cultures from Austrian winter peas. Characteristic nodule assemblies are more distinct in greenhouse samples than in field samples. (Fig. 2.) Table 8 shows that the average number of nodules per plant is considerably higher when poor cultures of Austrian winter peas and Louisiana vetch were used than when beneficial organisms were used. The close similarity of response of Austrian winter peas to nodule organisms from Louisiana vetch and from apparently parasitized Austrian winter peas is indicative of the origin of the unadapted strains present in the soils studied.

#### SUMMARY

Cultures of nodule bacteria from poor and vigorous Austrian winter-pea (*Pisum arvense*) plants from Jeanerette, La., have been compared with nodule bacteria from closely related legumes in field and greenhouse tests.

Drainage obtained by ridging the soil had but little effect, and lime and basic slag had practically no effect on the crop produced.

A favorable well-defined effect in quantity and quality of crop was evident in Austrian winter peas treated with efficient nodule bacteria in soil known to contain inefficient nodule-producing organisms.

Evidence has been presented which shows that the nodule organisms of the Louisiana vetch (*Vicia ludoviciana*) reduce the yield of Austrian winter pea on account of their unadaptability to this host.

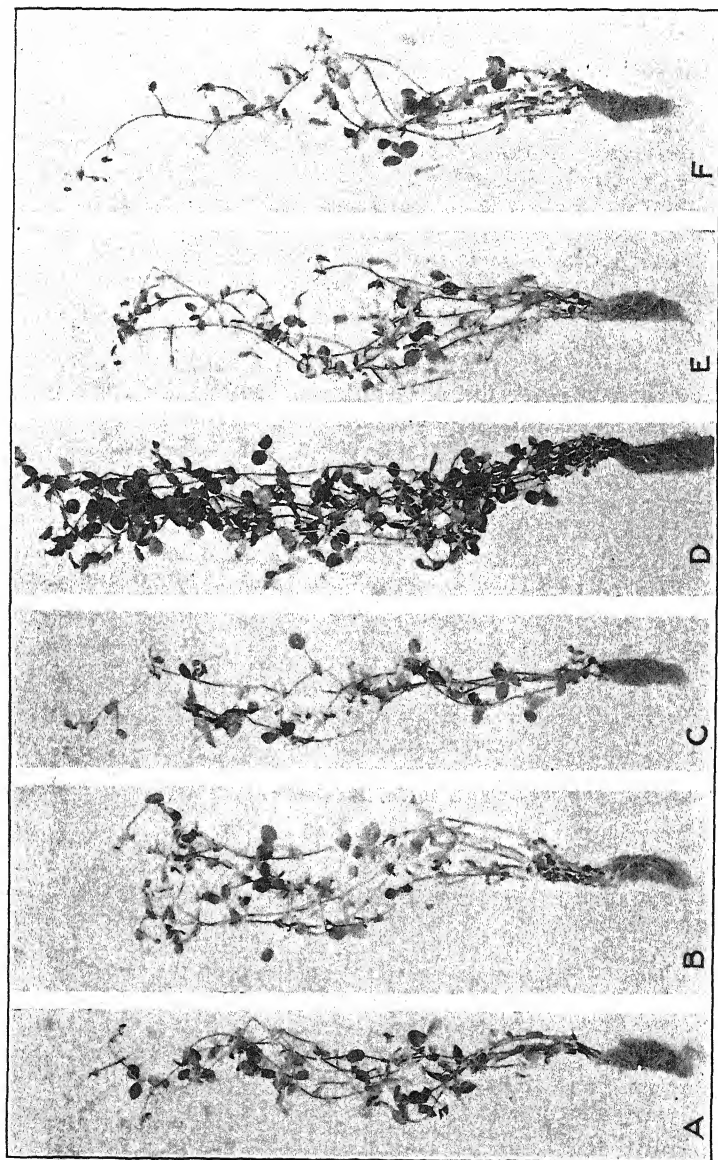


FIGURE 3.—Typical single-pot clumps of Austrian winter-pea plants treated with nodule organisms as follows: A, None; B, nonbeneficial culture 513; C, Louisiana vetch culture 548; D, beneficial culture 512; E and F, Louisiana vetch cultures 549 and 550

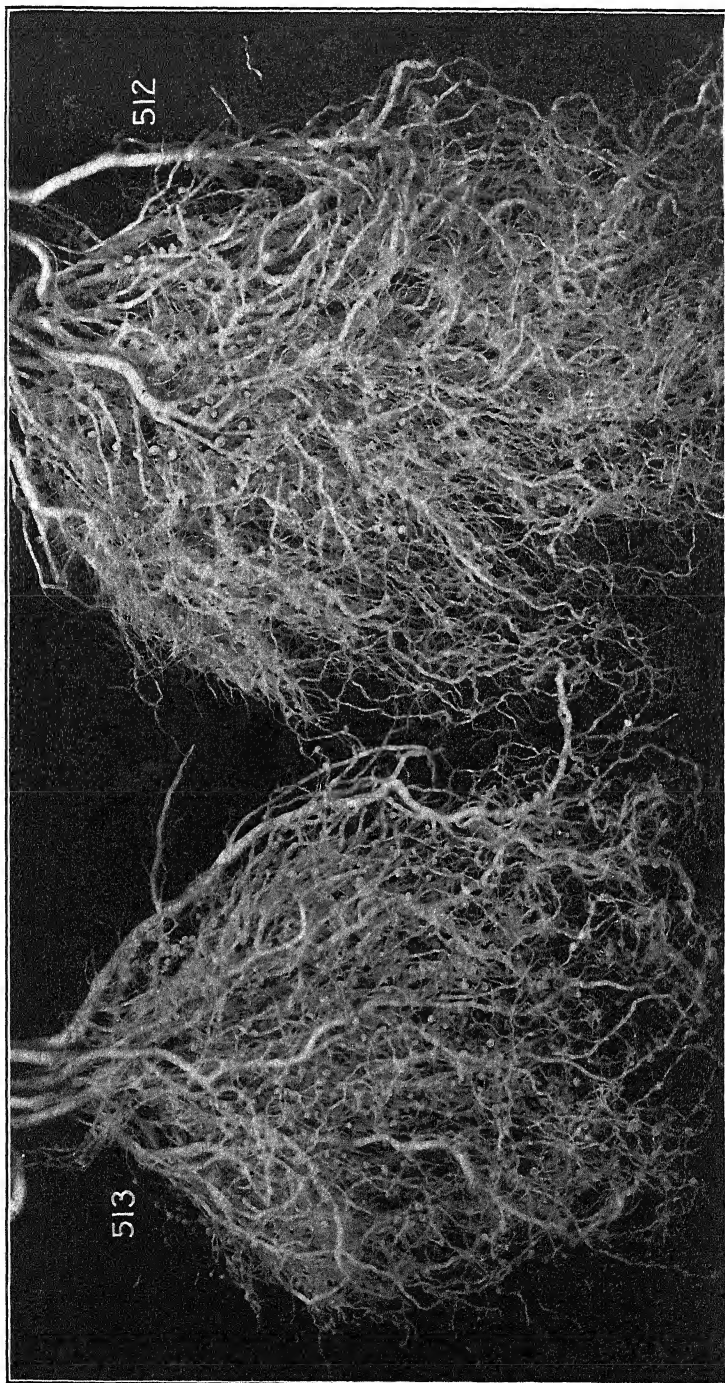


FIGURE 4.—Beneficial culture 512 and nonbeneficial culture 513 on Austrian winter peas grown in greenhouse

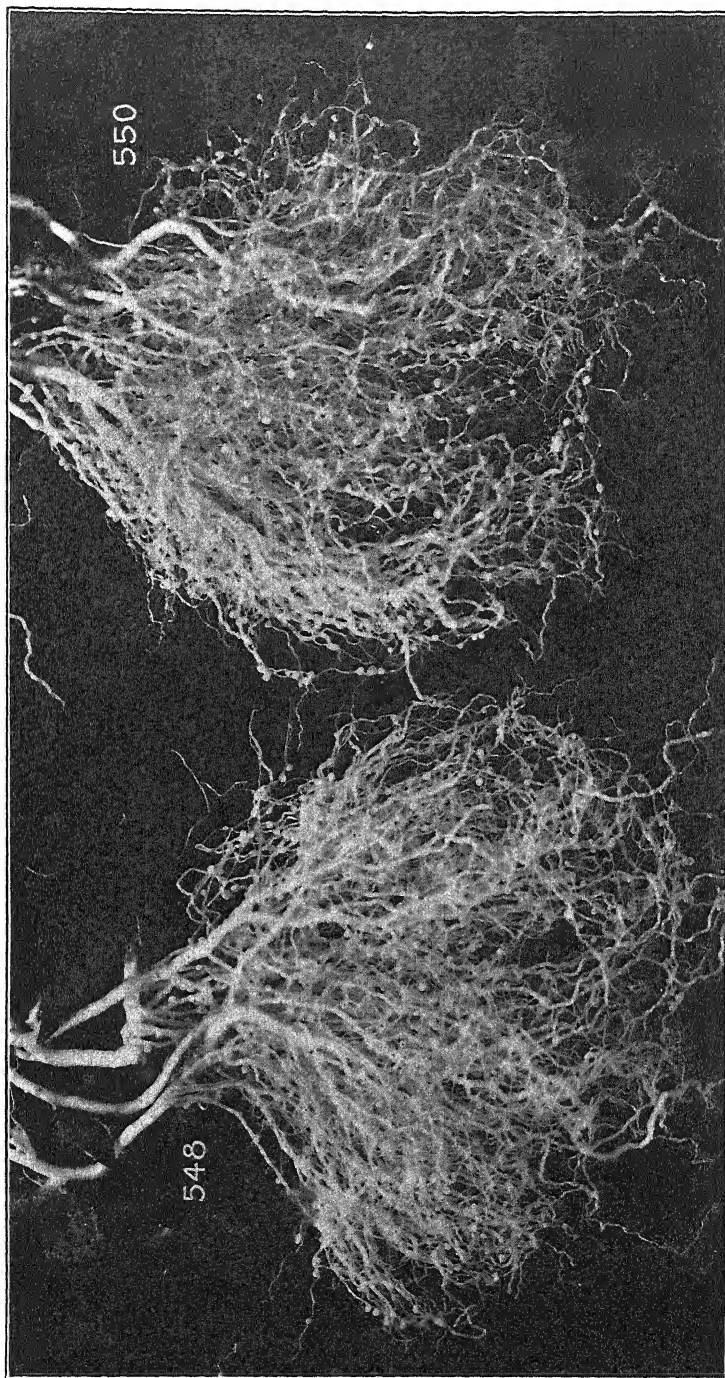


FIGURE 5.—Nonbeneficial types of nodulation produced by two different isolations from nodules of *Vicia ludoviciana* on Austrian winter pea in the greenhouse

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# CHEMICAL COMPOSITION OF NATIVE ALASKAN HAYS HARVESTED AT DIFFERENT PERIODS OF GROWTH<sup>1</sup>

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## INTRODUCTION

Alaska is handicapped agriculturally chiefly because of its very short growing season. However, some common vegetables, small fruits, cereals, and root crops are now being grown in many parts of Alaska, and certain native forage plants seem to thrive there. Most of these forage plants have a potential economic value. It was therefore deemed desirable to study the chemical composition of some of them cut at different stages of growth and to compare them with other forage plants cultivated in Alaska.

## PREVIOUS INVESTIGATIONS

In 1904 the Bureau of Chemistry (4, p. 276)<sup>2</sup> made analyses for the Alaska station of three native grasses which were cut when in flower. The results are given in Table 1. Extensive investigations of this general nature have been conducted at certain State experiment stations. Knight, Hepner, and Nelson (7), of the Wyoming Agricultural Experiment Station, studied the chemical composition of forage plants grown at different altitudes (where the growing season is short) and harvested at the same stage of growth. Hepner (5), also of the Wyoming station, investigated the relation of the nitrogen content of soils to the nitrogen content of grasses grown at high altitudes. Cundy (3) and Robert (8) continued these investigations on forage plants. The former studied the variation in the chemical composition of samples collected at different periods during the haying season, and the latter studied the effect of altitude, seasonal variation, and shade on their chemical composition. Hopper and Nesbitt (6), of the North Dakota Agricultural Experiment Station, likewise analyzed native prairie grasses at several stages of growth.

TABLE 1.—Composition (percent) of Alaskan forage plants<sup>a</sup>

Plant	Moisture	Ash	Ether extract	Crude fiber	Proteids	Nitrogen-free extract
Bluetop ( <i>Calamagrostis langsdorffii</i> ).....	7.18	3.90	1.03	42.94	4.58	40.37
Sedge ( <i>Carex cryptocarpa</i> ).....	5.85	10.65	2.12	25.72	10.32	45.34
Beach rye ( <i>Elymus mollis</i> ).....	11.92	7.51	2.26	30.31	12.71	35.29

<sup>a</sup> Georgeson, C. C. (4, p. 276).

<sup>1</sup> Received for publication Aug. 31, 1932; issued May 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 668.

## PRESENT INVESTIGATION

Sixteen samples each of native sedge (*Carex* sp.), redbtop (*Calamagrostis* sp.), and cotton grass (*Eriophorum* sp.) were recently submitted to this bureau by the Office of Experiment Stations for analysis, together with one sample each of native vetch (*Vicia cracca*), yellow-flowered alfalfa (*Medicago falcata*), and brome grass (*Bromus inermis*) to be used for comparison. These were all forage plants grown at the Alaska Agricultural Experiment Station at Fairbanks. The nomenclature has not been checked. The sedge, redbtop, and cotton grass were sampled or harvested at intervals of one week from July 22 to November 4, 1929, and the vetch, alfalfa, and brome grass were harvested on July 19, 1929.

The report of the Alaska Agricultural Experiment Stations (1) shows that during April, May, and June the weather was comparatively dry and cool, and plants grew slowly. During the second week of July there was a heavy rainfall, and growth was vigorous during the remainder of the month. August and the first part of September were cool, cloudy, and wet, causing crops to mature slowly, and harvesting in general was delayed until the last week in September.

The plants were air-dried when received. They were ground and analyzed for moisture, ash, nitrogen, fat, fiber, and sugars. The analytical methods used were those outlined by the Association of Official Agricultural Chemists (2, p. 277). The time of harvesting and the results of the analyses are shown in Table 2.

The protein content of the native forage plants was highest in the third cutting, that of August 5, and in the main decreased as the season advanced.

The fiber content gradually increased in all cases as the stage of growth advanced. This is especially true of redbtop.

The percentage of ash in sedge was fairly constant, increasing slightly until the last three cuttings, or the middle of October, after which it dropped appreciably. The ash content of redbtop remained fairly high throughout the entire period of growth; in fact, it was greater in the last cuttings than in the early ones. The ash of cotton grass was low and decreased as the season advanced, being similar in this respect to the ash of sedge.

The percentage of fat (ether extract) remained fairly constant in all samples harvested at different periods. In the sedge, the three latest cuttings had a slightly higher fat content than the early cuttings, whereas in redbtop the four earliest were higher in fat than the subsequent cuttings; cotton grass was more or less variable throughout.

The percentage of sugars was uniformly higher in the early cuttings. The late cuttings contained from 50 to 20 per cent of the amount of sugar found in the early cuttings.

The results show that at the same period of harvesting, the native sedge, redbtop, and cotton grass compare favorably with vetch and brome grass in protein content, but are considerably lower in this constituent than is the yellow-flowered alfalfa.

The fiber content of cotton grass and redbtop agrees fairly well with that of alfalfa and brome grass. The fiber of sedge is the lowest of all, and that of vetch is the highest.

TABLE 2.—Composition (per cent) of samples of Alaskan forage plants cut at various stages of growth

## NATIVE SEDGE (CAREX SP.)

Date cut (1929)	Moisture	Ash	Nitrogen	Protein	Fat	Crude fiber	Total sugar
July 22.....	5.26	4.36	1.76	11.00	1.79	28.95	3.82
July 29.....	5.41	4.72	1.31	8.19	1.74	32.71	5.42
Aug. 5.....	5.38	4.66	1.96	12.25	1.51	30.70	2.17
Aug. 12.....	4.98	4.34	1.74	10.88	2.17	30.00	3.40
Aug. 19.....	4.98	4.38	1.57	9.81	1.88	29.32	5.25
Aug. 26.....	4.85	4.78	1.32	8.25	2.02	25.55	5.70
Sept. 2.....	5.08	4.49	1.25	7.81	2.56	34.59	2.20
Sept. 9.....	4.96	5.32	1.39	8.69	1.45	34.30	1.90
Sept. 16.....	4.85	4.75	1.28	8.00	1.48	32.66	3.21
Sept. 23.....	5.62	4.58	.94	5.87	1.53	32.66	-----
Sept. 30.....	5.18	4.66	.91	5.69	1.34	34.12	.59
Oct. 7.....	5.54	5.58	1.01	5.31	1.56	35.87	-----
Oct. 14.....	5.38	5.20	.93	5.78	1.80	34.19	.86
Oct. 21.....	5.24	3.36	.59	3.68	2.18	35.87	.85
Oct. 28.....	4.78	3.22	.62	3.89	2.13	36.89	.57
Nov. 4.....	4.84	2.91	.59	3.69	2.00	35.66	.52

## REDTOP (CALAMAGROSTIS SP.)

July 22.....	5.33	4.47	1.42	8.88	1.78	32.85	3.17
July 29.....	5.13	4.41	1.71	10.69	1.74	31.51	4.87
Aug. 5.....	4.94	6.01	1.84	11.50	1.74	32.37	3.26
Aug. 12.....	4.86	7.24	.78	4.88	1.85	33.13	3.60
Aug. 19.....	5.04	5.09	1.00	6.25	1.33	36.51	4.80
Aug. 26.....	4.95	6.98	.74	4.63	1.67	34.73	3.29
Sept. 2.....	4.77	6.60	.56	3.50	1.69	35.51	4.04
Sept. 9.....	4.81	6.53	.81	5.06	1.72	36.98	3.50
Sept. 16.....	5.05	5.40	.56	3.50	1.66	39.22	2.74
Sept. 23.....	5.37	7.35	.81	5.06	1.59	34.85	2.36
Sept. 30.....	5.07	4.20	.74	4.63	1.42	38.21	.25
Oct. 7.....	4.58	4.41	.75	4.69	1.52	40.90	.45
Oct. 14.....	4.46	6.51	.43	2.63	1.56	40.99	.70
Oct. 21.....	4.70	6.64	.34	2.13	1.60	38.02	.26
Oct. 28.....	4.80	6.34	.36	2.25	1.49	40.61	.82
Nov. 4.....	4.81	6.77	.36	2.25	1.69	41.57	.06

## COTTON GRASS (ERIOPHORUM SP.)

July 22.....	5.94	2.93	1.54	9.63	1.90	31.96	2.45
July 29.....	5.52	2.17	1.47	9.19	1.59	31.45	5.88
Aug. 5.....	5.90	2.57	1.64	10.19	1.44	33.28	2.40
Aug. 12.....	5.62	2.48	1.57	9.81	1.38	32.30	3.54
Aug. 19.....	5.77	2.51	1.42	8.88	1.50	29.89	4.01
Aug. 26.....	5.98	2.50	1.40	8.75	1.86	27.77	5.73
Sept. 2.....	6.00	2.11	1.06	6.62	2.01	32.85	3.95
Sept. 9.....	5.44	1.80	.90	5.63	2.01	34.85	3.01
Sept. 16.....	5.55	2.03	.87	5.44	1.89	32.24	3.42
Sept. 23.....	5.48	1.72	.74	4.63	1.92	33.38	1.71
Sept. 30.....	5.64	1.63	.78	4.88	1.91	35.30	2.28
Oct. 7.....	5.55	1.90	.92	5.75	1.92	33.45	2.72
Oct. 14.....	5.80	1.68	.52	3.25	1.68	33.07	1.78
Oct. 21.....	5.85	1.97	.64	4.00	1.77	33.44	1.47
Oct. 28.....	5.86	1.91	.67	4.19	1.76	33.88	1.75
Nov. 4.....	5.69	1.79	.64	4.00	1.54	34.63	1.20

## VETCH (VICIA CRACCA)

July 19.....	6.60	6.65	1.71	10.69	1.40	35.54	1.32
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## YELLOW-FLOWERED ALFALFA (MEDICAGO FALCATA)

July 19.....	6.72	7.22	2.48	16.50	1.34	32.96	2.26
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## BROMEGRASS (BROMUS INERMIS)

July 22.....	5.51	7.29	1.22	7.63	1.38	32.71	5.76
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Vetch, brome-grass, and alfalfa are all considerably higher in ash and appreciably lower in fat than are cotton grass, sedge, and redtop. Sugar content is highest in brome-grass and lowest in vetch; the sugar content of alfalfa is about the same as that of cotton grass, but considerably lower than that of sedge and redtop.

#### SUMMARY AND CONCLUSIONS

Fifty-one samples of forage plants grown in Alaska in 1929 were analyzed for moisture, ash, crude protein, fat, fiber, and sugar.

The percentage of protein decreased as the season advanced. Redtop especially showed a markedly low protein content after the early stages of growth.

The percentage of fiber increased in all cases as the season advanced.

The ash content of sedge and redtop was higher than that of cotton grass at every stage of growth. The ash content of redtop was higher in the late cuttings than in the earliest ones. The reverse was true in the case of cotton grass and sedge.

The fat content of sedge and cotton grass was slightly higher than that of redtop.

The native plants examined were richer in sugar (at the same stage of growth) than the introduced plants, with the exception of brome-grass.

The native forage plants of Alaska on the whole compare favorably with vetch and brome-grass but not with yellow-flowered alfalfa.

According to these analyses the feeding values of the native hays decrease rapidly after August, as is shown by the decrease in protein and the increase in fiber. Therefore it would seem advisable to harvest these varieties late in August or early in September.

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# A CHEMICAL STUDY OF ENSILING SOYBEANS<sup>1</sup>

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## INTRODUCTION

In the course of an investigation of the feeding value of soybean silage, observations were made upon the normal changes that occur in soybeans during the ensiling process. These observations were made in an effort to determine the efficiency of the silo in preserving the feed nutrients of a legume roughage. Such information is of particular importance in regions where seasonal rainfall ordinarily prevents the satisfactory curing of hay.

## WORK OF OTHER INVESTIGATORS

Soybeans (*Soja max*) have been investigated extensively as a combination silage crop planted in conjunction with corn, cane, and other erect-growing forage crops, but only rarely have they been ensiled alone. Hills (4)<sup>2</sup> ensiled 564 pounds of green soybeans between layers of corn, and found that they produced a pleasant aromatic silage palatable to cattle. Eckles (3) mentioned losses as arising from surface exposure, mold, and the normal fermentation processes in the silo. These investigators, as well as Woll and Humphrey (9), reported comparative analyses of fresh soybeans and the silage made from them.

## PLAN OF THE INVESTIGATION

In each of three consecutive years (1929, 1930, and 1931) a silo 10 feet in diameter by 20 feet 10 inches in height was filled with soybeans cut in the early bloom stage, giving about 15 feet of settled silage. As the filling progressed large samples were taken, and 1 kg of each was oven dried for chemical analysis. Ten kilograms were placed in a muslin bag and returned to the silo. Five such bags were deposited at various depths in the center of the silo each year. Each bag was covered with a layer of silage over which a dampened piece of burlap was placed to aid in locating it when the silage was being removed.

When removed, the contents of each bag were weighed, and 1 kg was oven dried for chemical analysis. By a computation of the quantity of each constituent determined, both in fresh soybeans and in the corresponding sample bag, changes in composition incident to the ensiling process were determined. In making the determinations surface spoilage was not considered, its importance being related to the height of the silo.

Proximate analyses were made by the methods of the Association of Official Agricultural Chemists (1); and calcium, magnesium, and phosphorus by the method of Morris, Nelson, and Palmer (5).

<sup>1</sup> Received for publication Oct. 18, 1932; issued May, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 673.

## PRESENTATION AND DISCUSSION OF RESULTS

Fifteen parallel samples of fresh soybeans and of soybean silage were analyzed. The averages of these analyses are presented in Table 1. The analyses of other workers, computed to the dry-matter basis, are given for comparison. In general, these latter indicate a decrease in the nitrogen-free extract, slight change in the protein content, a relative increase in crude fiber and ash, and apparently an increase in ether-soluble compounds arising from the end products of the fermentation processes in the silo.

TABLE 1.—*Composition (percentage) of green soybeans and of soybean silage, as reported by the Florida, Missouri, Vermont, and Wisconsin Experiment Stations*

Source and class of feed	Total moisture	Composition on moisture-free basis				
		Crude protein	N-free extract	Crude fiber	Ether extract	Ash
Florida: <sup>a</sup>						
Fresh soybeans.....	74.61	12.12	40.00	36.38	2.41	9.07
Soybean silage.....	76.94	8.77	36.35	41.10	3.34	10.44
Missouri (3):						
Fresh soybeans.....	75.00	17.60	40.80	25.60	4.40	11.60
Soybean silage.....	76.00	18.33	34.17	26.67	8.33	12.50
Fresh soybeans.....	69.80	15.89	37.09	28.48	6.62	11.92
Soybean silage.....	62.10	16.36	44.06	26.39	4.49	8.71
Vermont (4):						
Fresh soybeans.....	75.36	20.29	41.56	22.53	3.46	12.16
Soybean silage.....	77.16	17.61	34.44	26.09	2.86	19.00
Wisconsin (9):						
Fresh soybeans.....	73.89	14.67	41.82	26.46	4.86	12.18
Soybean silage.....	73.92	15.68	33.70	30.44	8.05	12.12

<sup>a</sup> The Florida values are average analyses of 15 pairs of samples of Biloxi and Ootootan soybeans cut in the early-bloom and small-pod stages of maturity. All others are of earlier varieties harvested when more mature.

Variations in the ash content of the fresh soybeans and of the silage samples may be attributed to the relative loss of the other nutrients in the process of fermentation, migration of soluble ash in the expressed plant fluids, variations in the ash present in the plant tissues, or soil contamination in the original soybeans at the time they were ensiled. Too few samples are reported in the literature to afford conclusive evidence of changes occurring in the commercial ensiling of soybeans.

The writers' results shown in Table 1 indicate less protein and ether extract, and more crude fiber, than do similar analyses reported by other stations. The lower percentage of protein and higher percentage of crude fiber may be attributed to differences in the stage of maturity, variety, and loss of a part of the leafy and more succulent portions of the plant by insect damage. The smaller percentage of ether extract is due largely to the absence of beans.

The silage had less protein and nitrogen-free extract, and more crude fiber and ether extract than did the fresh soybeans. The apparent increase in crude fiber is due to its being least affected during the fermentation process. Ether-soluble hydrolytic products account for the increase in ether extract. The crude protein and nitrogen-free extract are the constituents most susceptible to hydrolysis during ensiling, volatile gases and water being the principal products. Some odor of protein putrefactive products was noticed from the surface spoilage, but practically none from the sound silage.

The soil on which these soybeans were grown was a Gainesville sandy loam (of residual phosphatic origin) having an admixture of clay. The soybeans contained over 1 per cent of calcium and about 0.4 per cent of phosphorus. The total ash was lower than that of other analyses reported in the literature. Other detailed mineral analyses have not been noted.

As previously stated, definite weights of freshly cut soybeans were placed in muslin bags and deposited at different levels in the silo. An average interval of 117 days elapsed from the date of ensiling the soybeans until the top sample bags were removed. The lower sample bags were removed after they had remained in the silo 147, 169, 183, and 191 days, respectively. Samples from each of these bags were analyzed. As the silo was emptied during the feeding trials, the contents of the individual bags were weighed, and samples were taken and analyzed. From the weights and analyses of the fresh soybeans and silage, the gains or losses of the various constituents of the soybeans in the bags have been computed. Since the bags were made of muslin, all volatile gases could pass off readily, and materials in solution could drain from or into the various bags; hence their contents were really a component part of the entire mass of silage.

TABLE 2.—Changes in composition during the ensiling process, based on actual amounts of dry matter in fresh soybeans and in bags of silage from different levels in the silo

#### FRESHLY CUT SOYBEANS

Level in the silo <sup>a</sup>	Net weight of fresh material	Composition on fresh moist basis									
		Moisture	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Calcium	Magnesium	Phosphorus
	<i>Kilo-grams</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Top.....	10.00	72.11	27.89	3.15	0.62	10.49	11.22	2.41	0.3088	0.1017	0.1119
Second.....	10.00	76.53	23.47	2.77	.53	8.24	9.41	2.52	.2607	.0825	.0900
Third.....	10.00	74.37	25.63	3.13	.64	9.44	10.41	2.01	.3044	.0941	.1078
Fourth.....	10.00	75.10	24.90	3.45	.59	8.80	9.93	2.14	.2819	.0895	.0992
Fifth.....	10.00	76.47	23.53	2.71	.65	8.67	9.21	2.29	.2729	.0928	.0916
Average..		74.92	25.08	3.04	.61	9.13	10.03	2.27	.2857	.0921	.1001

#### SOYBEAN SILAGE

Top.....	9.252	72.64	27.36	2.46	0.78	11.56	9.91	2.65	0.3296	0.1051	0.1042
Second.....	9.321	74.81	25.19	2.13	.86	9.66	9.12	3.41	.2880	.0958	.0892
Third.....	9.929	77.33	22.67	2.07	.73	9.31	8.42	2.14	.3084	.0903	.0990
Fourth.....	10.263	79.03	20.97	1.86	.65	9.03	7.67	1.77	.2560	.0849	.0848
Fifth.....	11.143	80.81	19.19	1.63	.66	7.92	6.89	2.09	.2369	.0819	.0839
Average..	9.982	76.92	23.08	2.03	.74	9.50	8.40	2.41	.2838	.0916	.0922

#### PERCENTAGE RECOVERY OF CONSTITUENTS OF FRESHLY CUT SOYBEANS IN SOY-BEAN SILAGE

Top.....	92.52	93.20	90.75	72.20	116.76	101.92	81.73	101.66	98.75	95.61	86.14
Second.....	93.21	91.11	100.06	71.84	151.90	109.30	90.36	126.06	102.95	108.24	92.30
Third.....	99.29	103.24	87.83	65.53	113.02	97.88	80.30	105.64	100.60	95.22	91.22
Fourth.....	102.62	107.99	86.41	55.32	112.43	105.34	79.25	84.76	93.21	97.36	87.74
Fifth.....	111.43	117.76	90.86	67.24	112.24	101.81	83.32	101.46	96.75	98.28	102.00
Average..	99.82	102.75	91.05	66.07	120.26	103.09	82.85	104.54	98.44	98.67	91.56

<sup>a</sup> The values given at each level are the average of one sample obtained during each of the 3 years' investigation.

The results of these computations based on samples at five different levels in the silo over 1 3-year period are presented in Table 2. The figures indicate that there was a decided gravitational movement of moisture as well as a slight actual increase. This increase can be accounted for by the moisture resulting from the hydrolysis of organic matter in excess of the very small evaporation. The loss of dry matter amounted to 9 per cent, largely from the protein and nitrogen-free extract. This is less than was observed by Brouwer (2) in grass silage, and nearly the same as was noted by Shaw, Wright, and Deysher (8) in corn silage. Peterson, Hastings, and Fred (7) found a 10 per cent loss of dry matter in corn-silage samples placed in water-proof bags to prevent loss or gain by gravitational movement of liquid.

A 17 per cent loss of nitrogen-free extract was observed, which is less, both actually and on a percentage basis, than that reported for corn silage in investigations summarized by Shaw, Wright, and Deysher (8). Acidity and sugars were not determined. However, the silage was mild, and tasted less sour than good corn silage. There was little change apparent in the crude fiber.

The greatest loss among the constituents studied was in the crude protein, which amounted to 34 per cent; the loss was less at the top and greater at the bottom of the silo. This rather exceptional loss may have been caused by more extensive bacterial action on proteins and less on carbohydrates, legumes having a smaller percentage of carbohydrates than corn. Moreover a part of this, apparent rather than real, might have been due to the escape of volatile nitrogenous compounds in drying the samples before analysis. It would seem that fermentation continued during all the time that the silage was being removed, leading to a loss of more nitrogen toward the bottom of the silo than was gained by the down wash of nitrogen in solution. The warmer climate of Florida and the lower acidity of a legume silage might be conducive to more prolonged surface fermentations.

The ether extract increased 20 per cent, probably as a result of the formation of additional ether-soluble substances. There was little down wash of the inorganic constituents in spite of the shift in gravitational moisture. Perhaps such movement, as has been observed by Shaw, Wright, and Deysher (8) and Perkins (6) in corn silage, might be observed with legume silage in a taller silo.

These changes here recorded in the composition of the ensiled soybeans are the result of the combined action of bacterial fermentation, plant enzymes, chemical interaction, and down wash of solutions in the silo.

#### SUMMARY AND CONCLUSIONS

Soybeans ensiled in the early bloom stage and having less than the usual (or recommended) dry-matter content made a mild and slightly aromatic silage.

There was a 9 per cent loss of dry matter, principally from the protein and nitrogen-free extract. The loss of protein was greater near the bottom of the silo, probably due to continued fermentation for a longer time. In crude fiber there was no loss, and in ether extract there was an absolute increase. On the whole the losses were comparable to those found by others in ensiled corn plants or grasses.



Gravitational movement of water and, to a lesser extent, of organic constituents was observed; gravitational movement of mineral matter was slight.

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## METHOD OF DETERMINING AGE OF BLISTER RUST INFECTION ON WESTERN WHITE PINE<sup>1</sup>

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### INTRODUCTION

Blister rust (*Cronartium ribicola* Diet.) was discovered in western North America in the fall of 1921. In 1922 it was found that the disease ranged over a wide area in southwestern British Columbia and adjacent Washington. Apparently it was originally introduced near Vancouver in 1910.<sup>3</sup> Its range now extends practically from Alaska to California and from the Pacific coast in Washington east into Montana. The chief aecial host within this range is native western white pine (*Pinus monticola* Doug.). Investigations of the rust in this great new range have been carried on by the Division of Forest Pathology since 1922. One of the first essentials in these investigations was to establish a method of determining the age of infection on pines. In the present paper this problem is discussed and a solution offered.

*Pinus monticola* occurs in two distinct geographic belts. One of these belts extends between the Pacific coast and the eastern slopes of the Coast Range in British Columbia, from about 150 miles north of the international boundary, southward into northwestern Washington, and thence mainly along the Cascade Mountains of Washington and Oregon into the higher elevations of the Sierra Nevada of California; the other belt extends from about 200 miles north of the international boundary, in the interior of British Columbia, southward into the main commercial range of the species in northern Idaho and adjacent Washington and Montana. Until 1929 the known range of pine infection by the blister rust was confined chiefly to the coastal belt in British Columbia and northern Washington and to a small portion of the interior belt 120 to 135 miles north of the boundary, in British Columbia. The range of infection is now known to extend, in the coastal belt, south in the Cascade Mountains to central Oregon, and, in the interior belt, into the main commercial pine region of Idaho. The results reported here are mainly from studies made within the earlier known portions of the range, which constitute the area of longest established and heaviest infection, but they are also

<sup>1</sup> Received for publication Aug. 19, 1932; issued May 1933.

<sup>2</sup> The writer was assisted in the collection of data on which this article was based by J. L. Mielke, T. S. Buchanan, A. A. McCreedy, J. W. Kimmey, and W. F. Cummins, of the Division of Forest Pathology, and C. N. Partington, formerly of that division, and by W. V. Benedict, of the Division of Blister Rust Control.

<sup>3</sup> PENNINGTON, L. H. RELATION OF WEATHER CONDITIONS TO THE SPREAD OF WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST. Jour. Agr. Research 30:598. 1925.

based on data from the newer sections of the range. The data from the two areas are in such close agreement as to indicate that the results will be applicable to the entire range of *P. monticola* in the Pacific Northwest.

#### CONDITIONS GOVERNING INFECTION OF WESTERN WHITE PINE

*Cronartium ribicola* infects its aecial hosts through the needles.<sup>4</sup> The needles of *Pinus monticola* are regularly held for three full growing seasons. Although some needles fall at the end of the third season, a great many are generally held through the fourth season, and some are commonly held to the end of the fifth season. If the growth rates of the needle-bearing parts and the environmental conditions are favorable, a few needles may be retained through the sixth or even the seventh season, and very exceptionally a needle bundle or two may persist through the eighth season. Thus infection may conceivably find entrance to the bark on internodes of any age up to eight seasons. It is obvious, on the other hand, that the best chances for entrance are on the younger growth and that the dropping of the needles prevents much infection from entering on internodes more than 4 seasons old.

Infection of the needles may occur at any time that moisture conditions are favorable during the period in which sporidia are produced. For all practical purposes, in the range of *Pinus monticola* this may be considered as limited to the period during which teliospores are produced; or, roughly, from about June 1, when these spores first begin to form, until about November 30, when *Ribes* plants have lost their leaves and most of the fungus spores have germinated.

Ordinarily the majority of the teliospores are produced within a relatively short period. This concentration of telial sporulation is particularly marked, and comes earliest in the pine-infection centers where *Ribes* plants are plentifully infected by aeciospores early in the season. Under these conditions the teliospores are usually produced in maximum quantities, and their production reaches its height in July or August.

Any favorable moist period during the time when teliospores are abundant results in the production of great numbers of sporidia and, if other conditions are favorable, in extensive infection on pines. On the other hand, if conditions are not favorable, the delicate sporidia, through ineffective germination or loss of viability, soon lose all power to infect, and thus the pine-infecting potentialities of the fungus on *Ribes* are largely dissipated for the season. The present investigations have shown that for the range of *Pinus monticola* in general conditions are favorable for heavy pine infection on an average of one season out of three.

#### DEVELOPMENT OF THE CANKERS

Infection is followed by an incubation period during which the rust grows through the needle into the bark, where its further development causes sufficient discoloration or swelling to permit of an accurate macroscopic diagnosis.<sup>5</sup> This period varies for individual infections.

<sup>4</sup> CLINTON, G. P., and McCORMICK, F. A. INFECTION EXPERIMENTS OF PINUS STROBUS WITH CRONARTIUM RIBICOLA. Conn. Agr. Expt. Sta. Bul. 214: [428]-459, illus. 1919.

<sup>5</sup> CLINTON, G. P., and McCORMICK, F. Op. cit., p. 449-455.

Observations of the rust through its western range on *Pinus monticola* indicate that the first discoloration or visible swelling of the bark that marks the incipient canker appears only under exceptional circumstances in the first season following that of infection, that it appears principally in the second season, to a lesser degree in the third season, and to a negligible degree, if at all, later than the third season.

The period required for production of the fruiting stages varies also.<sup>6</sup> When the incipient canker makes its appearance early or during the middle of the season, pycnia are generally formed upon it the same year. Cankers appearing toward the end of the season usually do not produce pycnia until the next year. On the majority of the cankers aecia are formed in the year following that in which pycnia are produced, but on many not until the second year.

For young cankers it is seldom difficult to determine the year's growth (wood<sup>7</sup>) on which they originated, and on thrifty trees the internodes of entrance can usually be determined for cankers of much greater age. The age of the growth on which the canker originated readily establishes the maximum possible age of the canker, since it is obvious that infection can not occur prior to the formation of the part infected. Considered in relation to the general appearance of the canker, this affords a rough index of its age. But in the great majority of cases it is impossible to determine accurately the year of infection for the individual canker. Only by considering large numbers of cankers classified according to the year's growth upon which they occur and to their stage of development can the year of infection be determined.<sup>8</sup>

### CANKER PRODUCTION

Since the teliospores in pine-infection areas are usually produced within a relatively short period during any season, the occurrence of heavy infection of the pines will necessarily be correspondingly restricted in point of time, possibly to a few days toward the end of the period of teliospore production, or immediately following it. As might be expected, therefore, incipient canker formation in the infection centers generally occurs in definite waves, and usually the majority of the cankers of the individual waves show a marked tendency to form simultaneously.

Heavy waves of canker formation occurred in most of the infection-study areas of the coastal section in 1922 and 1923, in the interior section in 1924, and more or less generally throughout the range in 1929. In 1922 most of the cankers appeared in midseason, during late July and August. In 1923 the greatest numbers appeared in June and July. Most of those produced in the interior study areas in 1924 developed from midseason until September, although some continued to form until the end of the season. In 1929 concentrated production generally occurred toward the end of the season and in some places started up for a time the following spring.

<sup>6</sup> SPAULDING, P. INVESTIGATIONS OF THE WHITE PINE BLISTER RUST. U. S. Dept. Agr. Bul. 957:24-27. 1922.

<sup>7</sup> The term "wood" has been used by others in a sense similar to that for which the present writer prefers the term "growth," to designate internodes of given years.

<sup>8</sup> PENNINGTON, L. H. Op. cit., p. 596.

Most of the heavy waves of canker formation and some of less intensity have stood out distinctly, in some cases strikingly so. For example, at an infection area near Revelstoke, British Columbia, where the rust was discovered on pines in 1922 and had clearly been present on them for a number of years, no more than 48 cankers, incipient or otherwise, could be found on them until 1924. In June of that year a wave of incipient canker formation began, reaching such proportions in July and August that by September incipient and juvenile cankers could be counted in the area by the thousands. At another area near Revelstoke, where infection had been present on the pines since 1922, the number of cankers increased more than 1,000 per cent during the latter part of the growing season in 1929 and the spring of 1930.

#### DISTRIBUTION PATTERN OF CANKERS IN INCIPIENT CANKER WAVES

The incipient cankers of the waves of canker formation that occurred in 1922 maintained a highly regular numerical arrangement in their distribution on the internodes of different years. The youngest growth on which these cankers occurred was that of 1920. Most of the cankers, however, were concentrated on the growths of 1919 and 1918, the greatest number being on 1919 growth. Their occurrence was relatively light on 1917 growth, infrequent on 1916 growth, and very exceptional or lacking on older growths. Since these incipient cankers had appeared almost simultaneously, it seemed probable that the majority, at least, had resulted from a common infection year.

Inasmuch as some of the cankers occurred on 1920 growth, it was evident that the majority must have resulted from an infection not earlier than that of 1920. Since none was present on growth younger than 1920, this year was evidently the infection year. If the infection had occurred in 1921 some of the cankers would undoubtedly have been present on 1921 growth; if the infection had occurred in 1922, there would not have been sufficient time for even incipient cankers to develop, or assuming incubation to have taken place, the cankers would have appeared on the growth of 1922 as well as on that of other years.

The waves of canker formation that occurred in 1923 showed a distribution closely analogous to that of the 1922 waves. No cankers were found on 1923 or 1922 growth. Relatively few were found on 1921 growth; the great majority occurred on 1920 and 1919 growth, and the greater number of these on 1920 growth; 1918 growth had relatively few, and older growths were very little affected. The same general relations have characterized the distribution of cankers of each distinct wave of canker formation since then.

It is evident that this characteristic numerical distribution of the cankers, combined with similarity in stage of development, provides a means of ascertaining the infection years for groups of cankers in more advanced stages, thus making it possible to determine the main years of infection and to work out the history of the rust from analyses of older classes of cankers.

For convenience in presenting and summarizing the observations and data the following symbols are used:

- a*, youngest growth upon which cankers of the infection wave were found.  
*b*, growth 1 year older than *a*.  
*c*, growth 2 years older than *a*.  
*d*, growth 3 years older than *a*.  
*e*, growth 4 years older than *a*.  
*f*, growth 5 years older than *a*.  
*g*, growth 6 years older than *a*.  
*h*, growth 7 years older than *a*.

Figure 1 shows diagrammatically these symbols and the normal occurrence of needles for several successive years.

Most of the needles on *d* in A are dropped at the end of the season, and the remainder are generally dropped at the end of the season the following year. The chances of infection through the needles on

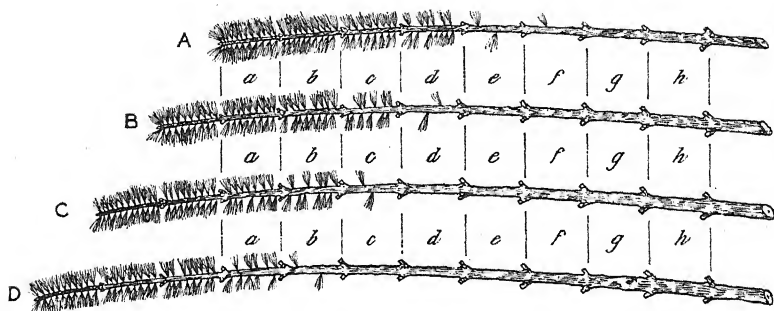


FIGURE 1.—Needle retention in *Pinus monticola* infected with *Cronarium ribicola*: A, Branch after completion of internode *a*; B, branch one year later; C, branch two years later (when most of the cankers appear); D, branch three years later (when the majority of the remaining cankers appear); *a*, youngest growth, or internode, on which cankers of the infection wave were found; *b* to *h*, growths, or internodes, 1, 2, 3, 4, 5, 6, and 7 years older than *a*, respectively

older growths are obviously small. Undoubtedly this accounts for the small representation of cankers on such growths shown in the characteristic pattern of the cankers of the distinct waves of canker formation, which is summarized below:

Growth	Cankers
<i>a</i> -----	Relatively few.
<i>b</i> -----	Greatest number
<i>c</i> -----	Considerable numbers
<i>d</i> -----	Relatively few.
<i>e</i> -----	Very few.
<i>f</i> -----	Exceptional.
<i>g</i> -----	None.
<i>h</i> -----	None.

Sample counts of cankers from these waves are presented in Table 1; the number on each year's growth is expressed as a percentage of the total.

The individual records consistently show the same general relationships. The percentage of cankers on *a* is normally relatively small, averaging about 10; *b* and *c* together have consistently the largest percentage (80-90), with much the largest percentage (usually more than 50) on *b*; the percentage on *d* is generally relatively small (averaging about 5); and that on older growths is practically negligible.

TABLE 1.—Canker records from distinct waves of canker formation<sup>a</sup> in various areas

## COASTAL REGION, BRITISH COLUMBIA

No.	Elevation	Location	Youngest growth showing cankers (a) <sup>b</sup>	Year of canker formation	Number of cankers	Percentage of cankers on--						
						a <sup>c</sup>	b	c	d	e	f	g
1		Thurston Bay	1920	1922	77	17	52	23	7	1	0	0
			1920	1922	53	10	58	28	4	0	0	0
			1920	1922	68	6	50	43	1	0	0	0
			Total or average		198	11	53	31	4	1	0	0
			1921	1923	76	12	63	13	9	3	0	0
			1921	1923	81	14	60	20	6	0	0	0
			1921	1923	136	7	57	34	2	0	0	0
			Total or average		293	10	59	24	5	1	0	0
			1922	1924	123	19	61	17	3	0	0	0
			1924	1926	115	9	55	34	1	1	0	0
			Total or average		729	12	57	26	4	1	0	0
			2		Pender Harbor	1921	1923	134	5	54	37	4
1920	1922	40				8	41	35	8	0	0	0
3	Near sea level	Chee Kye	1921	1923	417	8	49	37	6	0	0	0
			1921	1923	302	10	53	32	5	0	0	0
			Total or average		719	8	51	35	6	0	0	0
			1922	1924	48	17	64	19	0	0	0	0
4		Sperling	1923	1926	45	16	60	22	2	0	0	0
			Total or average		852	9	52	33	6	0	0	0
			1921	1923	91	7	55	31	7	0	0	0
			1923	1925	56	11	57	30	2	0	0	0
5		Qualicum Beach	1924	1926	112	7	68	21	4	0	0	0
			1927	1929	40	12	55	30	9	0	0	0
			Total or average		208	9	63	25	3	0	0	0
			Total or average		2,014	10	55	30.2	4.6	.2	0	0

## COAST MOUNTAINS, BRITISH COLUMBIA

6	Feet 1,300	Daisy Lake.....	1921.....	1923-24	98	10	56	24	10	0	0	0
7	800	Owl Creek.....	1923.....	1925	145	2	53	43	2	0	0	0
			1924.....	1926	123	4	60	33	2	0	0	0
			1927.....	1929	52	8	48	34	10	0	0	0
			Total or average.....		320	4	55	38	3	0	0	0
8	1,300	Mile 72, P. G. E. Ry....	1920.....	1922	121	14	42	33	10	1	0	0
			1921.....	1923	124	13	46	34	4	1	0	0
			Total or average.....		245	13	44	35	7	1	0	0
			Total or average.....		663	8	51	34.5	6	.5	0	0

<sup>a</sup>From main body of wave where possibility of admixture of cankers of two successive years was least in each case. Samples were taken indiscriminately through the stand from trees that could be examined from the ground or easily climbed. Cases indeterminate in regard to age of growth infected or stage of development of the canker were not included.

<sup>b</sup>From sample records of the Division of Blister Rust Control.

<sup>c</sup>For definition of symbols a, b, c, d, etc., see page 679.



TABLE 1.—Canker records from distinct waves of canker formation<sup>a</sup> in various areas—Continued

## OTHER AREAS

No.	Elevation	Location	Youngest growth showing cankers (a) <sup>b</sup>	Year of canker formation	Number of cankers	Percentage of cankers on—						
						a <sup>b</sup>	b	c	d	e	f	g
9	1,500	Revelstoke, British Columbia.	1922.....	1924	296	7	52	31	8.5	1.5	0	0
			1922.....	1924	307	3	51	34	10	2	0	0
			1922.....	1924-25	180	14	52	27	6.5	.5	0	0
			Total or average.....		783	7	52	31	8.7	1.3	0	0
10	2,000	Columbia River, 8 miles north of Revelstoke.	1927.....	1929-30	1,805	12	54	30.12	3.5	.33	.05	0
11	1,700	Arrow Park, British Columbia.	1927.....	1929	135	8	53	36	3	0	0	0
12	3,100	Apex, British Columbia, experiment plot.	1928.....	1930	144	5	52	32	10	1	0	0
13	2,500	Mount Hood, Oreg., near Rhododendron.	1927.....	1929	92	9	42	37	12	0	0	0
14	500	Eagle Creek, Oreg.....	1927.....	1929-30	243	14	42	36	7	1	0	0
			Total or average.....		3,202	10.2	52.3	31.3	5.5	.67	.03	0
			Total or average.....		5,879	9.8	53.2	31.3	5.21	.47	.02	0

See footnotes on p. 680.

## DETERMINATION OF YEAR OF INFECTION

The typical distribution pattern of the incipient cankers, their simultaneity of appearance, and the conditions governing infection of the pines may be taken as conclusive evidence that the majority of the cankers of each wave are approximately the same age and of common origin; i. e., that they resulted from an infection of the same year. On this basis, the year of infection for each of these waves is determined by the youngest growth affected. In most cases this is the growth that formed two seasons before the appearance of the cankers. In the waves that became apparent in 1922, for example, the youngest growth showing cankers was that of 1920; in the waves of 1923, it was the growth of 1921; in the 1924 waves, it was the growth of 1922, etc.

Since there may be some doubt as to these conclusions, however, the analysis of the 1922 waves may be carried somewhat further. It may be assumed that infection occurred in 1921. Since none of the cankers were present on 1921 growth, it would follow either that the needles of 1921 were immune to infection or that a longer time was required for incubation in the needles and bark of 1921 than in the needles and bark of older growths, and that the infection on 1921 growth had not yet become apparent. Since relatively few of the cankers occurred on 1920 growth, it would be necessary to assume either that the needles of 1920 were resistant to infection in 1921 or that incubation was slower for this growth than for older growths. In accordance with the regular habit of needle retention in *Pinus monticola* (fig. 1), 1919 growth (b), which was in its third season of development in 1921 (fig. 1, B), lost some of its needles at the end of the season in 1921. Most of the needles that remained on 1918 growth (c) fell at the same time. Yet the great majority of the

cankers occurred on 1919 and 1918 growths. Therefore, for 1918 growth at least, the rust must have grown through the needles and into the bark with extraordinary rapidity. The growth of 1917 (*d*) was practically devoid of needles in 1921. (Fig. 1, B.) Yet the number of cankers on this growth, although small in comparison to the number on 1919 and 1918, growth was actually very considerable. Since most of the few needles remaining on this growth were cast in the fall of 1921, the only possible explanation would be that some of the infection on this growth occurred directly through the bark, which was then 4 seasons old. Certainly this explanation is too improbable to merit further consideration, and it is obvious that to assume that infection occurred in 1922 would require an explanation still more improbable.

If it is assumed that the cankers of the 1922 waves resulted from the combined infection of two or more years, it would be necessary to conclude, since these waves exhibited the typical arrangement of distinct canker-formation waves generally, that this arrangement characterized the distribution of cankers of two or more infection years forming simultaneously and that all the distinct waves arose from similar combinations, for the typical arrangement is too consistently maintained to be accidental. On this basis, for example, if it is assumed that the 1922 waves resulted half from 1919 infection and half from 1920 infection, then the 1923 waves must have originated from equal proportions of infection of 1920 and 1921, the 1924 waves from equal proportions of infection of 1921 and 1922, and the 1929 waves from equal proportions of infection of 1926 and 1927. In other words, the infection years would necessarily bear a regular proportional relationship toward one another, which is obviously beyond probability.

Evidently, then, it is reasonable to accept the typical arrangement as evidence that the majority of the cankers of any one of these waves resulted from infection in a single year and that the youngest growth affected (*a*) marks the infection year; i. e., it is the growth formed the year infection occurred. Thus Table 1, column 4, gives the infection year for each of the waves of which the cankers were counted.

The dates of the infection as determined by the foregoing methods have been further confirmed by observations of weather conditions. In the years shown by the analyses of cankers to have been heavy infection years, conditions have been favorable for heavy pine infection; and when analyses have shown that the infection was light, conditions have been unfavorable for infection.

The method of age determination is thus supported by a complete chain of evidence comprising the following series of phenomena found occurring repeatedly and uniformly throughout a wide range of representative areas over a period of nine years of observations:

- (1) Evidence or actual observations of outstanding favorable seasonal conditions for infection of the pines.
- (2) Two seasons later a heavy wave of canker formation, sometimes extending into the third season.
- (3) Distribution of these cankers in accordance with a characteristic distribution pattern.
- (4) Only one acceptable explanation of the canker distribution, namely, that most of the cankers in each wave were the same age; i. e., that they resulted from an infection of the same season.

(5) Date of the infection season fixed by the distribution of the cankers as a favorable season two or three seasons before the appearance of the majority of the cankers.

Since, however, before the appearance of the canker the infection year is indicated only by its outstandingly favorable conditions in sporidia production and moisture, the evidence for the hypothesis herein advanced is primarily circumstantial. It is desirable, therefore, to consider cases where there could be no doubt in regard to the year of infection. The opportunity to do this occurred recently in a case in which the infection year was definitely known before the cankers appeared. In this case two *Ribes* plants, the only specimens standing in a small isolated area of sound pine, were infected artificially in 1928. *Ribes* infection was not present in the area in 1927 or in 1929. In the growing season of 1930, 144 incipient cankers appeared only on trees within a short distance of the *Ribes* and in greatest profusion on those in the immediate vicinity of the *Ribes*. There can be no doubt that these cankers resulted from the infection on the *Ribes* plants in 1928. The record of these cankers (Table 1, area No. 12) follows closely the general trend and average of the other records.

It has been stated earlier in this paper that cankers are rarely found on trees in the first season after the year of infection. This is the case with most trees. On trees more than 3 feet high and 8 years old the writer in nine seasons of observations has never seen an incipient canker on growth younger than that formed two years before the year of examination. On the youngest seedlings and on those up to about 5 years old, however, the writer has positive proof that a great many if not most of the cankers appear in the season following that of infection. In determining the heavy infection years, therefore, cankers on such seedlings must not be included with the records of infection on the older trees, which generally constitute most of the growth in any natural infection area and therefore have the great majority of the cankers.

Variation in needle retention is another factor that must be taken into account in investigations of the history of infection in any pine area. The normal period of needle retention described earlier in this paper does not apply absolutely, for in areas where growth is relatively slow, usually at higher elevations, the needles are commonly held for a somewhat longer period than is normal; and where growth is particularly rapid they are actually held for a somewhat shorter period than is normal. This is especially noticeable on the faster growing parts such as the leaders and upper branches of thrifty trees, where the needles are commonly shed a full year earlier than is normal.

Since infection occurs through the needles, it is clear that the distribution of the cankers on growths of different years will be influenced by differences in needle retention. It may be assumed, for example, that areas 8, 9, 12, and 13, in Table 1, represent conditions of high altitude and slow growth and that areas 1 and 5 represent conditions of lower altitude and particularly rapid growth. Table 2 shows the two scales of arrangement for the two types of conditions separately. The arrangement for the grand total of Table 1 may for all practical purposes be considered normal; the data are given as approximate percentages for comparison in Table 2.

TABLE 2.—Percentage of cankers found on growths of different years under conditions of low altitude and rapid growth and of high altitude and slow growth

Growth affected	Percentage of cankers under indicated conditions			Growth <sup>a</sup> affected	Percentage of cankers under indicated conditions		
	Low altitude (rapid growth)	High altitude (slow growth)	Normal		Low altitude (rapid growth)	High altitude (slow growth)	Normal
<i>a</i> .....	10+	7+	10—	<i>f</i> .....	0	0	Trace.
<i>b</i> .....	60	49+	53+	<i>g</i> .....	0	0	0
<i>c</i> .....	26—	34—	31+	<i>h</i> .....	0	0	0
<i>d</i> .....	3+	9+	5+	<i>i</i> .....	0	0	0
<i>e</i> .....	0.5	1—	0.5—				

<sup>a</sup> Growth of season of infection; i. e., youngest growth showing cankers of the wave.

As might be expected, the high-altitude group shows a greater spread of the infection to older growths, *c* and older growths having more than the normal amounts and *a* and *b* growths less; whereas the low-altitude scale shows more infection than normal on *b* growth and less than normal on *c* and older growths. As a rule, the differences are small, and the same general relationships prevail in all the scales.

#### TECHNIC FOR DETERMINING AGE OF CANKERS

The application of the foregoing scales of arrangement is simple. On encountering a new infection area the investigator first determines the general span of needle retention in order to decide what scale to apply in determining the infection years. The normal scale or even the typical distribution as expressed in descriptive terms alone (p. 679) will usually suffice for general application. Records should then be made of the cankers classified according to stage of development. The following classification has been found most useful:

##### Young cankers:

*First symptoms*.—First signs of infection on the bark; incipient cankers indicated by discoloration less than one-half inch in length on the smaller, slower-growing twigs and branches and up to about three-quarters inch in length on the larger branches and leaders, usually showing little or no swelling.

*Juvenile*.—Small infections that have developed beyond the stage of first symptoms but have not yet produced pycnia.

*First pycnia*.—Infections that at the time of the count or earlier in the same season were bearing pycnia for the first time.

*Pycnial scars*.—Infections that had borne pycnia for the first time the preceding year but failed to produce aecia in the spring of the year in which the count was made.

*First aecia*.—Infections that bore aecia for the first time in the spring of the year in which the count was made.

##### Old cankers:

*Aecia produced twice*.—Cankers that bore aecia for the second season in the spring of the year in which count was made.

*Aecia produced several times*.—Cankers that had borne aecia for several seasons.

*Dead*.—Old cankers in which death prevented further development.

From first to last, these headings represent normal progressive steps, following incubation, in the development of the individual infection.

In order to apply this scheme of classification it is necessary to consider two facts: (1) That cankers grow longitudinally at an average rate of approximately 2.5 to 3 inches per year on twigs and branches less than one-quarter inch in diameter, and (2) that this rate increases

up to an average of about 7 or 8 inches a year on parts 3 inches or more in diameter. It is also necessary to be familiar with the normal development of fruiting stages described on page 677. Where fruiting has obviously been retarded, the plan of classification may be enlarged to include such cankers.

In Table 3 an illustration is given of the application of the distribution pattern to the analysis of the history of the rust in a typical infection area near sea level. Table 3 gives a count taken indiscriminately on April 27, 1924, from the cankers found on thrifty trees ranging from approximately 10 to 30 years of age. The count included only cankers for which it was possible to determine definitely the age of the growth on which the canker started and the stage of development of the canker.

TABLE 3.—Canker tabulation, Chee Kye, British Columbia, April 27, 1924

Growth affected	Number of cankers at indicated stage of development							
	Young					Old		
	First symptoms	Juvenile	First pycnia	Pycnial scars	First aecia	Aecia produced		Dead
						Twice	Several times	
1	2	3	4	5	6	7	8	9
1924.....	0	0	0	0	0	0	0	0
1923.....	0	0	0	0	0	0	0	0
1922.....	0	0	0	0	0	0	0	0
1921.....	12	9	0	4	19	0	0	0
1920.....	19	21	0	23	162	3	0	0
1919.....	2	10	0	16	126	20	0	0
1918.....	0	0	0	0	27	14	2	0
1917.....	0	0	0	0	0	3	2	0
1916.....	0	0	0	0	0	0	0	0
1915.....	0	0	0	0	0	0	2	0
1914 and older.....	0	0	0	0	0	0	0	0

The count did not include cankers of the first year of infection on pines in this area. Special search for older cankers showed, on growth ranging from that of 1910 to that of 1913, a few very old cankers. The arrangement of these old cankers and the fact that weather records indicate 1913 to have been an outstandingly favorable year for infection of both Ribes and pine, showed that the cankers probably resulted from infection in 1913. The analysis of the older cankers, not included in Table 3, showed considerable intensification in 1917 and 1918, particularly in the latter year. A comparison of the normal distribution pattern with the tabulation in Table 3 shows that much intensification occurred in 1920 and a very large amount in 1921. From the distribution relationships of the cankers listed in columns 2 and 6 of Table 3 it is evident that the great majority are of 1921 origin. The proportion of these cankers to older cankers, in view of the fact that the number recorded represents but an infinitesimal fraction of the number present, indicates that a great wave of intensification occurred in 1921. Practically all the cankers in column 7, from their arrangement, are clearly of 1920 origin.

Table 4 gives a tabulation for the same area from November 15 to 16, 1924, the year in the spring of which the tabulation in Table 3 was made. The same rules applied in making the tabulation given in Table 3 were observed in making the tabulation given in Table 4.

TABLE 4.—Canker tabulation, Chee Kye, British Columbia, November 15 to 16, 1924

Growth affected	Number of cankers at indicated stage of development							
	Young					Old		
	First symptoms	Juvenile	First pyrenia	Pycnial scars	First aecia	Aecia produced		Dead
						Twice	Several times	
1	2	3	4	5	6	7	8	9
1924.....	0	0	0	0	0	0	0	0
1923.....	0	0	0	0	0	0	0	0
1922.....	6	1	3	0	0	0	0	0
1921.....	22	8	21	0	29	0	0	0
1920.....	4	5	24	22	137	8	0	0
1919.....	0	0	2	14	83	19	0	0
1918.....	0	0	0	1	16	7	3	2
1917.....	0	0	0	0	0	0	13	0
1916.....	0	0	0	0	0	0	6	0
1915.....	0	0	0	0	0	0	1	0
1914.....	0	0	0	0	0	0	1	0
1913 and older.....	0	0	0	0	0	0	0	0

A comparison with the normal distribution pattern shows clearly that considerable additional intensification of infection on pines occurred in 1922. The cankers in columns 2 to 4 of Table 4 are mainly of 1922 origin. Their number is small in proportion to the number of 1921 cankers (columns 5 and 6), but they seem to exceed the number in 1920 (column 7). It must be remembered, however, that as the cankers grow older they are increasingly difficult to classify exactly according to stage of development and year of origin. Therefore, since doubtful cases are not included in the counts, some allowance must be made to balance this difference. Observations indicated that cankers of the 1920 wave were slightly more abundant than those of the 1922 wave. In view of the fact that aecial sporulation and the opportunities for the infection of *Ribes* were much better in 1922 than in 1920, owing to the development of sporulating cankers between these years, it was evident from the count, together with the observations, that 1920 was a considerably more favorable year than 1922 for infection in this area.

As already indicated, canker development in *Pinus monticola* takes place chiefly in the second year after the year of infection and to some extent in the third year. The cankers generally begin to form in the spring of the second year and are produced in the greatest numbers during the summer of that year. In the winter, growth practically ceases in the host. Consequently, if all the cankers are made their appearance in the second year, the remainder do not develop until after active growth is resumed the following year.

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Normally the proportion of cankers produced in the third year is small and the period of formation is confined to the first months of spring. Frequently, however, a considerable number, sometimes many, are produced, and the period of formation continues until well into the summer.

Where successive infection years occur, the third-year production of cankers frequently results in an overlapping in the formation of the cankers of one infection year with that of the next and the intermixture of incipient cankers of two infection years. Since the proportion of cankers produced in the third year is usually small and production is generally limited to spring, the overlapping and intermixture are correspondingly slight and limited to spring; but when third-year production is considerable and prolonged the overlapping and intermixture may be extensive.

Besides occurring at incipency, intermixture of cankers normally takes place during the later stages because of variation in rate of development and production of fruiting stages and the consequent impossibility of classifying the cankers according to the year of origin after they have passed the early stages. Thus, cankers formed in the fall of one year do not produce pycnia until the following summer. Meantime cankers resulting from the next infection year are formed in the spring and produce pycnia at the same time as do the cankers formed the preceding fall. The result is an intermixture of the cankers of the two consecutive infection years in the "First pycnia" column. If the count is taken shortly before the formation of pycnia, there will be an intermixture of the two sets under the "Juvenile" classification.

The frequency of intermixture increases with increasing age. For example, not all the cankers that produce first pycnia one year produce aecia the following spring; some cankers normally produce their first aecia the second spring after the production of pycnia. Where successive infection years occur this will result in considerable intermixture under the classification "First aecia." The extent and complexity of intermixture will obviously be still greater among the older cankers.

This intermixture, whether it occurs at incipience or later, tends to obscure the actual distribution relationships of the cankers. Fortunately, the infection years in the earlier stages of the epidemic are generally separated by periods of one to several years during which little or no infection occurs; if later they become successive they usually are highly unequal, so that the years of heavy infection stand out. But even if intermixture is extensive the infection years may be differentiated by use of the typical distribution relationships.

The application of the relationships in these cases may be best illustrated by a hypothetical example. Assuming that 100 cankers of each of two successive infection years, 1922 and 1923, represent a single stage of development and that in both cases the distribution of the cankers is identical with the normal scale, it follows that the distribution of each set and of the sums of the two sets, the latter constituting the numbers recorded, would be approximately as given in Table 5.

TABLE 5.—*Hypothetical distribution of cankers in a case of intermixture of 100 cankers from each of two infection years*

Growth affected	Number of cankers			Growth affected	Number of cankers		
	From 1923 infection	From 1922 infection	Total		From 1923 infection	From 1922 infection	Total
1923.....	10	0	10	1918.....	0	1	1
1922.....	53	10	63	1917.....	0	0	0
1921.....	31	53	84	Total.....	100	100	200
1920.....	5	31	36				
1919.....	1	5	6				

Application of the scale to the combined totals in this case would permit an almost exact segregation of the two sets. Obviously such an equal combination and exact agreement of the individual components with any distribution scale is not to be expected in nature. But as already shown, there is consistently a sufficiently close adherence to the general relationships of the typical distribution pattern to permit analysis where intermixture occurs.

The detailed data collected by the Division of Blister Rust Control on the Long Meadow Creek and Three Bear Creek infection area, Idaho, in July, 1930, represent an extensive admixture caused by overlapping of canker formation and by variation in development, and illustrate a practical application of the distribution pattern to actual conditions. In this case the overlapping of canker formation and consequent intermixture in the cankers of the younger stages were extreme. (Table 6.) The area may be classified as normal in general conditions. But since most of the trees were of considerable height and the majority of the cankers were situated on the slow-growing branches of the lower crowns, which formed the major portion of the substratum for infection in close proximity to the Ribes, the scale of arrangement for slow-growing conditions was used in the analysis of the data.

TABLE 6.—*Canker tabulation, Long Meadow Creek and Three Bear Creek infection area, Idaho, July, 1930*

Growth affected	Number of cankers at indicated stage of development							
	Young					Old		
	First sym- ptoms	Juve- nile	First pycnia	Pyc- nial scars	First aecia	Aecia produced		Dead
						Twice	Several times	
1	2	3	4	5	6	7	8	9
1930.....	0	0	0	0	0	0	0	0
1929.....	0	0	0	0	0	0	0	0
1928.....	60	127	2	4	1	0	0	1
1927.....	111	750	35	61	65	0	0	3
1926.....	125	1,328	123	232	248	2	2	2
1925.....	39	578	73	146	304	6	7	2
1924.....	4	88	16	54	101	24	10	7
1923.....	0	12	1	10	11	4	7	3
1922.....	0	1	0	1	0	0	4	4
1921.....	0	0	0	0	0	0	0	1
1920 and older.....	0	0	0	0	0	0	0	0



A detailed analysis of the data in Table 6 gives the following results:

The distribution of the cankers in column 2 indicates that they are primarily of 1928 origin, although the inclusion of a certain proportion originating from 1927 infection is suggested by the relatively large representation on 1926 growth. Column 3 contains the majority of all the cankers counted. A superficial examination of the figures indicates that many of the cankers originated from infection in 1928 but that the greatest number originated from infection in 1927. This may be seen by comparing the great numbers of cankers on 1926 and 1925 growths with the typical distribution pattern as expressed in descriptive terms alone (p. 677). A more critical analysis may be made by trial and comparison of assumed values on the basis of the relationships shown in the scale. In column 3 the 127 cankers on 1928 growth are obviously of 1928 origin. Assuming the occurrence of cankers of this year on older growths in this column to be approximately 610 on 1927 growth, 500 on 1926 growth, 100 on 1925 growth, and 10 on 1924 growth, a total of 1,347 cankers is obtained, leaving a remainder of 1,537 distributed as follows: 140 on 1927 growth, 828 on 1926 growth, 478 on 1925 growth, 78 on 1924 growth, 12 on 1923 growth, and 1 on 1922 growth. In terms of percentage the distribution for the 1928 group becomes: *a*, 9; *b*, 45; *c*, 37; *d*, 8; *e*, 1; and for the 1927 group: *a*, 9; *b*, 54; *c*, 31; *d*, 5; and *e*, 1. The figures show that about 82 per cent of the cankers in the 1928 group and 85 per cent of the cankers in the 1927 group occurred on *b* and *c* growths, which compares well with the normal pattern and at the same time balances the variations from the values of the high-altitude scale as closely as is necessary for practical purposes. The conclusion is the same as that derived from the superficial analysis; i. e., that many of the cankers originated from infection in 1928 but that the greater number originated from infection in 1927.

In column 4 of Table 6 it is evident that the great majority of the cankers originated in 1927, although a few of 1928 origin are included also. Since 2 of the cankers occur on 1928 growth, this column, in accordance with the high-altitude scale of arrangement, should contain about 14 cankers of 1928 origin on 1927 growth, 10 on 1926 growth, and 2 on 1925 growth. This makes a total of about 28 cankers of 1928 origin, leaving a balance of 222 distributed as follows: 9+ per cent on 1927 growth, 51+ per cent on 1926 growth, 32+ per cent on 1925 growth, 7+ per cent on 1924 growth, and less than 1 per cent on 1923 growth. A comparison of the high-altitude scale with the distribution pattern of these cankers shows an almost exact agreement of the figures with the scale and definitely determines 1927 as the infection year for the group.

In column 5 of Table 6 is found a class of cankers that made their appearance and produced pycnia in the year preceding that of the tabulation but failed to produce aecia in the tabulation year, 1930. It will be noted that four cankers in this column were found on 1928 growth. These cankers, therefore, originated from 1928 infection and made their appearance in the following season, 1929. Since this is extremely unusual, except on young seedlings up to about 5 years old, it is presumed that these cankers were recorded from such seedlings, of which undoubtedly there was a certain representation on the area. The distribution of cankers resulting from a given year's infection on such young trees differs somewhat from the normal distribu-

tion on older trees and averages about 15 per cent on *a* growth, 69 per cent on *b*, 14 per cent on *c*, and 2 per cent on *d*. According to this relationship, since 4 of the cankers occur on 1928 growth column 5 should contain about 18 such cankers on 1927 growth and 4 on 1926 growth, making a total of about 26 cankers that may be considered to have originated from infection of 1928. There remain 482 cankers, distributed as follows: 9 per cent on 1927 growth, 47+ per cent on 1926 growth, 30+ per cent on 1925 growth, 11+ per cent on 1924 growth, 2+ per cent on 1923 growth, and less than 1 per cent on 1922 growth. Comparison of the arrangement of these cankers with the high-altitude scale shows that the infection year was 1927.

The majority of the cankers in column 6 of Table 6 are of 1927 origin, although a few are of 1928 origin, and there is evidently a very considerable proportion of 1926 origin on 1926 and older growths in this column. This is clear not only from the arrangement of the cankers but from the fact that in general the production of aecia occurs only in part of the cankers in the year following that of their appearance, aecial production on most of the remainder occurring in the following year.

The distribution of the cankers in column 7 of Table 6 indicates infection in both 1926 and 1925. The number of 1926 cankers in column 7 is only a fraction of the number in column 6. Evidently, then, most of the cankers that originated from 1926 infection either failed to produce aecia in 1929, following the production of first pycnia in 1928, or did not reach the stage of pycnial production until 1929, or both, and did not arrive at the stage of aeciospore production until the spring of 1930.

The distribution of the cankers in column 8 of Table 6 indicates infection in both 1926 and 1925. If the 1926 cankers in column 8 had actually produced aecia more than twice it would appear that they must have formed and produced pycnia at least by 1927, the year following infection. As already stated, this is a rare occurrence except on very young trees. Since the proportion of such cankers in this case appears to be rather large and since at the time of year the count was made cankers that have produced aecia only twice may easily appear to have produced aecia more than twice, it is more probable that part of the 1926 cankers in column 8 had actually produced aecia only twice and should have been included in column 7.

The prevalence of cankers on 1924 growth, as shown in columns 7 and 8 of Table 6, indicates that the larger proportion originated from infection in 1925. The number of 1926 cankers recorded in column 6, however, is greater than the total of all cankers in columns 7 and 8 and, together with the 1926 cankers in these two columns, indicates a decidedly heavier infection in 1926 than in 1925.

From a recapitulation of the foregoing analyses and from a broader consideration of the data in Table 6, two years—1927 and 1928—stand out as years of very heavy infection, 1927 showing much the heavier infection. In considering the degree of infection indicated by the record for the year 1928, however, it should be remembered that part of the cankers do not become apparent until the third year after the infection year. The tabulation was made in 1930. The incidence of cankers of 1927 origin in the youngest classes in stage of development indicates that in the Long Meadow and Three Bear

Creek area there is a considerable lag in incubation and that relatively large proportions of the cankers may not make their appearance there until the third year after the infection year. On this basis, a considerable proportion of the cankers of 1928 origin might not appear until 1931. Therefore, since the record was made in 1930, the indications of the degree of infection in 1928 must necessarily be considered incomplete.

In addition to the infection of 1927 and 1928, the analysis shows a certain amount of infection in 1925 and 1926, the greater amount in 1926.

The earlier infection history of the area had to be studied by means of special counts of the oldest cankers that could be found. The arrangement of these cankers and their situation on the area indicated that an appreciable amount of infection occurred in several local foci in 1923. No cankers were found that could be assigned to any earlier infection year. The infection in 1923 and that in 1925, when the 1923 cankers were just making their appearance but had not yet produced aecia, was considerably heavier and more profusely distributed than is normal through infection of *Ribes* by the long-distance spread of aeciospores from the outside. The usual ratio of *Ribes* infection to that on pines indicated that at least one or two sporulating cankers were present in the near vicinity at the time. It seems probable, therefore, that the original invasion of the locality by the rust must have occurred some years earlier than 1923.

From the data secured by these special counts and from the analysis of the tabulation in Table 6 the infection history for this locality may be summarized as follows:

- (1) Original invasion probably a few years before 1923.
- (2) 1923.—Very few aeciospores; favorable infection season; infection of pines at several points in the area.
- (3) 1924.—Very few aeciospores; unfavorable infection season; negligible intensification on pines.
- (4) 1925.—Very few aeciospores; favorable infection season; appreciable intensification of infection on pines.
- (5) 1926.—Great increase of aeciospores resulting from sporulation of the cankers of the infection of 1923; unfavorable infection season; considerable intensification on pines.<sup>9</sup>
- (6) 1927.—Great majority of 1923 cankers produced aeciospores; aeciospore production probably doubled or trebled that in 1926; very favorable infection season; great intensification on pines.
- (7) 1928.—Another large increase of aeciospore production as a result of beginning of sporulation on cankers of 1925 origin; favorable infection season; further heavy intensification on pines.

From the foregoing analysis it is evident that the application of the relationship of arrangement is essential to any determination of infection years where cankers of successive infection years are mixed. Pennington<sup>10</sup> has stated that—  
when a large number of cankers of approximately the same stages of development are found distributed through the internodes of three or four successive years, it is practically certain that infection resulted from exposure in the last of these years.

<sup>9</sup> As a result of the beginning of sporulation of the cankers of 1923 origin the number of aeciospores produced on the area must have been increased many hundredfold over that in 1925. Although the intensification of infection of pines in 1926 was about five or six times greater than that of 1925, the fact that it was much less than might have been expected from the increase of aeciospores and the opportunities for infection of *Ribes* on the area indicates that infection conditions in 1926 were unfavorable.

<sup>10</sup> PENNINGTON, L. H. Op. cit., p. 596.

The application of the method of determining the age of the cankers, as reported in this paper, to similar distributions shows that in general this is true; but one would be at a loss on this basis to determine the infection years when, as is commonly the case, the distribution normally covers internodes of five years or when the cankers of two infection years are mixed. Even when the cankers found are confined to growths of only four years, such mixtures may occur, as is indicated from the analysis of column 7 of Table 6. A comparison with the distribution pattern is therefore necessary for accurate diagnosis in any case. Where the infection years are as badly mixed as those in Table 6, such a comparison constitutes the only means of differentiation. With its aid it is possible, even in such cases, to obtain a good picture of the heavy infection years and of the history of the progress of the rust on pines.

### SUMMARY

*Pinus monticola*, in its natural range in the Pacific Northwest, generally casts most of the needles of any one year at the end of the fourth season, although some are held five seasons and a few may persist for as long as eight seasons. Since *Cronartium ribicola* infects its aecial hosts through the needles, infection in any season may conceivably find entrance to the bark on internodes up to 8 seasons of age but will necessarily enter chiefly on those of the last four seasons.

Conditions favorable for heavy pine infection in the Pacific Northwest are generally limited to a short period in any season and occur, on an average, about one season out of three. Heavy infection of pines in any year and locality usually becomes manifest in a more or less distinct wave of incipient canker formation in the second season following the season of infection. The cankers of any year of infection show a highly characteristic numerical relationship in their proportional distribution on the growths, or internodes, of different years. The characteristic relationship may be summarized as follows:

Growth affected	Number of cankers
a. Growth of the season of infection . . .	Relatively few.
b. Growth 1 year older than a . . . . .	Greatest number
c. Growth 2 years older than a . . . . .	Considerable number
d. Growth 3 years older than a . . . . .	Relatively few.
e. Growth 4 years older than a . . . . .	Very few.
f. Growth 5 years older than a . . . . .	Exceptional.
g. Growth 6 years older than a . . . . .	None.
h. Growth 7 years older than a . . . . .	None.

Thirty-two sample counts, totaling 5,879 cankers from distinct infection waves of five different years in 14 different places, showed the following scale of distribution for the total:

Growth	Percentage of cankers
a. . . . .	10—
b. . . . .	53+
c. . . . .	31+
d. . . . .	5+
e. . . . .	0.5—
f. . . . .	Trace.
g. . . . .	0
h. . . . .	0

In the individual counts, *b* and *c* together had consistently 80 to 90 per cent of the cankers, with much the largest percentage (usually more than 50 per cent) on *b*. The percentages on *a* and *d* were relatively small, *a* generally having the larger. The percentages on older growth were almost negligible.

A comparison of the typical distribution pattern with comprehensive sample records of cankers classified according to stage of development and year's growth affected will generally permit an accurate determination of the main infection years and provide a reliable guide to the progress of the rust on pines in any infection area.

Although incipient canker formation from a given year's infection is normally concentrated in the second season after that of infection, it may carry over extensively into the third season. This frequently results in an overlapping in the formation of the cankers and the consequent intermixture of cankers of two successive infection years under one classification in stage of development. Since the rate of development of the individual cankers varies, this intermixture of cankers becomes more common as development advances. The application of the distribution pattern is therefore essential for the accurate determination of infection years.



# SOME STORAGE AND TRANSPORTATIONAL DISEASES OF CITRUS FRUITS APPARENTLY DUE TO SUBOXIDATION<sup>1</sup>

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## INTRODUCTION

Before the investigations of Powell (15),<sup>3</sup> Stubenrauch, Ramsey and Tenny (20), and Ramsey (16) shipments of citrus fruits were subject to the hazard of excessive decay by fungi, especially of the *Penicillium* type. Rigid application of the principles adduced by these investigators has reduced to a minimum the losses from fungous decays formerly so excessive in transcontinental shipments. The need for further information concerning a group of diseases affecting oranges and grapefruit is frequently emphasized by the conspicuous deterioration that occurs in storage and transportation in the absence of fungous decay. These diseases, unlike the fungous rots, can not be traced to improper handling of the fruit, for they often occur in shipments handled under careful supervision, and in the highest quality brands.

Prior to investigations begun at Michigan State College in 1919, observations during 1917-18 of hundreds of cars of oranges and grapefruit from California, Florida, and Cuba at the Chicago, New Orleans, and Memphis terminals revealed the importance of these diseases. These observations were subsequently extended by numerous inspections of shipments at Detroit from 1919 to 1921. This paper presents the results of experiments on the effect of environmental conditions on the incidence of these blemish diseases, and some tests with chemicals and oiled wrappers in the production and prevention of these diseases.

The major part of this investigation pertains to storage spot or "pox" disease of orange and grapefruit. Some observations and experiments are included on brown spot of navel oranges and brown stain, a disease affecting oranges mainly in cold storage and marine shipments.

## EARLIER OBSERVATIONS

That citrus fruits are inherently subject to deterioration under cold-storage conditions was determined by comparatively early investigations. Stubenrauch (19) observed that oranges showed external evidence of deterioration in the form of brown spots, pits, and stains after four weeks in cold storage at 32°-42° F., but that no disease developed at 60°-65° in cellar storage.

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<sup>2</sup> The writer is indebted to Dr. E. A. Bessey for a critical reading of the manuscript and translation of the Italian reference.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 712.

Brown spot of Washington navel orange was first described by Smith (17, p. 47-48), but Coit (2) conducted the first extensive investigations; which, however, failed to disclose the nature and cause of the disease. Later observations by Smith and Smith (18, p. 1143-1145) confirmed the previous conclusions concerning the nonparasitic origin of brown spot, but marked no advance in the actual knowledge of the nature and cause of the blemish.

Ramsey (16) observed the deterioration of citrus fruits in cold storage and recommended the employment of temperatures considerably above 32° F.; that is, for oranges, 38°-40°; for lemons, about 42°; and for grapefruit, 45°-50°. He noted particularly the development of brown stains and spots on oranges at 32° and a serious spotting of grapefruit at the same temperature.

In a study of the changes occurring in Florida grapefruit in storage, Hawkins and Magness (9) found that a pitting of the rind developed at 40° F. or lower. Hawkins (7) determined that the amount of pitting could be greatly reduced by storage for one to three weeks at 70°. Later Hawkins and Barger (8) recommended treatment with kerosene-stove gas as a partial control of pitting.

Some transportational and storage diseases of vegetables were described by the writer (13) and shown to be due to oxygen deficiencies, and some surface blemishes of citrus fruits were similarly classified.

In their investigations of citrus fruit exported from South Africa, Thompson, Putterill, and Hobson (22) found it necessary to employ temperatures above 43° F. to prevent brown stain on oranges.

The earlier observations and experiments have shown that brown spot, brown stain, and storage spot are diseases associated with fruit stored at low temperatures or under conditions of poor ventilation. Although these three blemishes are seemingly distinct maladies, it is evident that they are closely related and are to be classified with a group of disorders among which apple scald is the classic example. The breakdown of citrus fruits at low temperatures is not a unique response limited to citrus. Tropical plants in general are apparently more subject to cold injury than those grown in temperate regions. The spotting of tropical and some other plants when shifted from warm to cold conditions has been noted by several investigators (Molisch (12); Harvey (6)). A progressive increase in the permeability of the cells of the endocarp of the orange has been shown by Pantanelli (14) to occur at low temperatures and indicates the injurious effect of cold on the protoplasm of citrus fruits.

#### ECONOMIC IMPORTANCE

Since brown spot, storage spot, and brown stain are diseases that do not cause decay, but merely spoil the appearance of the fruit and consequently lower the grade, the actual monetary losses traceable to these blemishes are difficult to estimate. In some seasons the aggregate losses from these diseases to producers and handlers of citrus fruits represent a sum too large to be disregarded. Fruit showing the effects of these blemishes is not only reduced in value but also may influence the general market prices for sound fruit. A few cars of spotted oranges or grapefruit arriving at a terminal may quickly depress prices and impede sales.



As early as 1909, Coit (2) observed the serious effects of brown spot on navel oranges in transcontinental shipments. He cited, a depreciation of \$307.20 in a car shipped from Riverside to Chicago on December 5, 1909, which on arrival there was diverted to Boston. The development of brown spot or storage spot in this car while moving from Chicago to Boston reduced the market value of the fruit 50 per cent. The total elapsed time from picking to sale was three weeks.

Just after Thanksgiving in 1920, the writer saw two cars of Florida grapefruit and 500 cases of navel oranges sold at the Detroit terminal for slightly more than transportation charges. All of this fruit was affected with storage spot. (Pl. 1.) Fruit arriving through December and into January of the same season was severely spotted but later little or no disease was apparent until April, when some shipments were again badly affected. Similar examples might be cited for other terminals and seasons.

Oranges affected with brown spot and oranges and grapefruit showing the effect of storage spot have been observed each year on the Lansing and Detroit markets. Usually fruit shipped during November and December is most seriously affected, but sometimes summer and late-season shipments are badly spotted. (Pls. 1, C; 2, D.) Although spotted fruit may be observed on the markets each year, seasonal variations in the incidence and severity of the blemishes influence the conspicuousness and consequent decline in the value of the fruit.

Fawcett and Lee (3) reported much cold-storage spotting in eastern markets during July and August 1924, on grapefruit held in cold storage for 5 to 12 weeks. Bratley <sup>4</sup> observed that California Valencia oranges arriving on the New York market in 1931 were more severely affected with spots and pitting than during previous years.

## SYMPTOMS

### STORAGE SPOT OR POX

The first symptom of storage spot on citrus fruits is the occurrence of slightly sunken spots of variable size over the surface of the fruit. (Pl. 3, B.) Inspection shows, in most cases, that the cells surrounding the oil glands are the first to be involved, these cells collapsing and leaving the oil glands standing out in relief above the sunken tissue. (Pls. 4, E; 3, B.) The lesion may increase in size for 24 hours or more, but usually its limits are determined by the initial collapse of the cells after exposure of the fruit to conditions favorable for the production of the disease. The spots may be so small that the area of collapsed tissue embraces only the cells surrounding a single oil vesicle, or the lesions may be several centimeters in diameter. (Pl. 1, B.) Often the diseased areas exceed one-half the surface of the fruit. (Pl. 1, B and C.)

The lesions at first are typically angular (pls. 1, C; 3, B), but eventually they assume a more or less circular outline (pl. 1, A and B). Secondary extension by fungi of a newly formed lesion seldom occurs, but old spots are sometimes invaded. As the lesions age the oil

<sup>4</sup> BRATLEY, C. O. NEW YORK MARKET PATHOLOGY NOTES. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rpt. 15:133. 1931. [Mimeographed.]

glands also collapse through desiccation of the adjacent cells, forming a depressed area with a sharply defined, darker border. The oil vesicles, when fully dried out, are sometimes more deeply sunken than the adjacent parenchyma cells. (Pl. 1, B.) In exceptional cases the cells over the oil glands are first affected and become depressed below the surface level of the fruit before the surrounding cells collapse.

The affected tissue is colorless at first but gradually changes to various shades of brown. The most common color of the grapefruit lesion is russet vinaceous.<sup>5</sup> On oranges the spots show a greater variety of color gradations, usually ranging from Natal brown to several vinaceous tints. The lesions are darker in the early-shipped fruit, and the color becomes less intense as the season advances until on spring and summer shipments of grapefruit and late shipments of navel oranges the spots may be almost colorless. (Pl. 1, C.) The lesion is always more deeply colored at the border, but this may not be apparent in the early stages of development. The darker color of the border is usually some shade of purple.

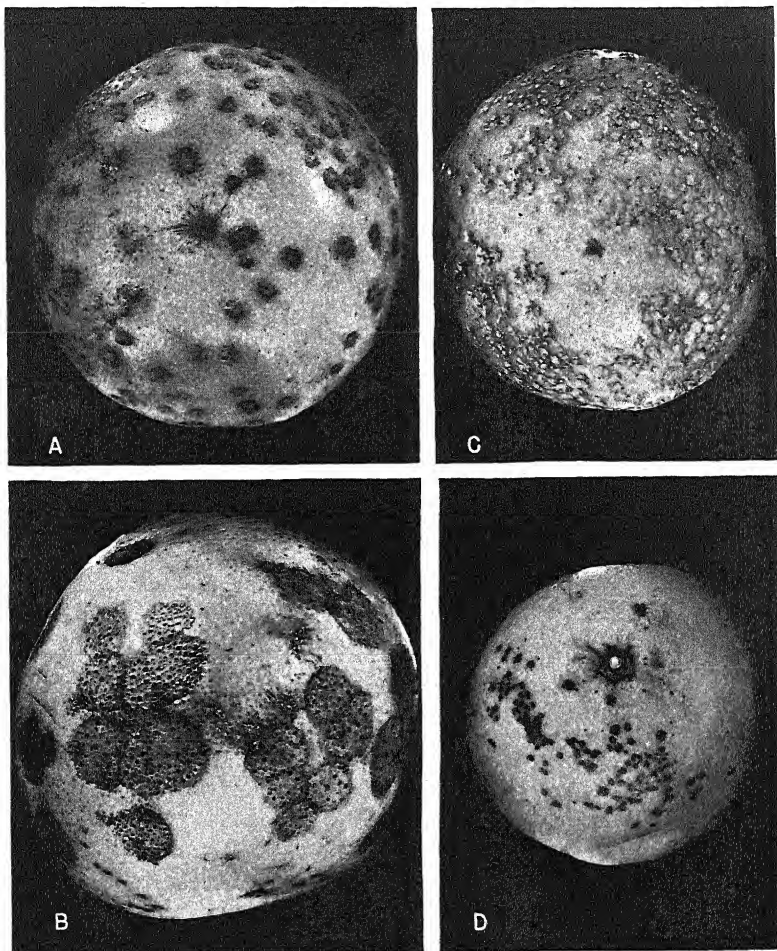
When examined in refrigerator cars, oranges and grapefruit may show no evidence of pitting, but if delay has occurred in transit or the fruit has been left in the car for some time after arrival, spotting may become evident. In cars frequently diverted breakdown may increase greatly in amount and severity and the fruit be seriously reduced in value before it reaches the market. Fruit held four to eight weeks at temperatures close to 32° F. develops characteristic lesions while still in storage. Unlike apple scald, which does not complete its development until the fruit is removed to a warm place, storage spot may become evident in two to four weeks, but the color typical of the lesions does not always develop until the fruit is removed to a higher temperature.

Histologically the changes that occur in the affected areas have their origin in the collapse of the parenchyma cells that surround the oil vesicles. The anatomical features of the normal peel are shown in Plate 4, A, and the general appearance of sections through storage-spot lesions on grapefruit are illustrated in Plate 4, B-D. The initial collapse of the active parenchyma cells around the oil glands is followed by the gradual desiccation of the affected cells and the formation of a depressed lesion. The edges of the lesions are bordered by a zone of deeper-colored cells also in a state of collapse. (Pl. 4 C and D.) In these cells there is a pigment that is absent from the other portions or is present in smaller quantities.

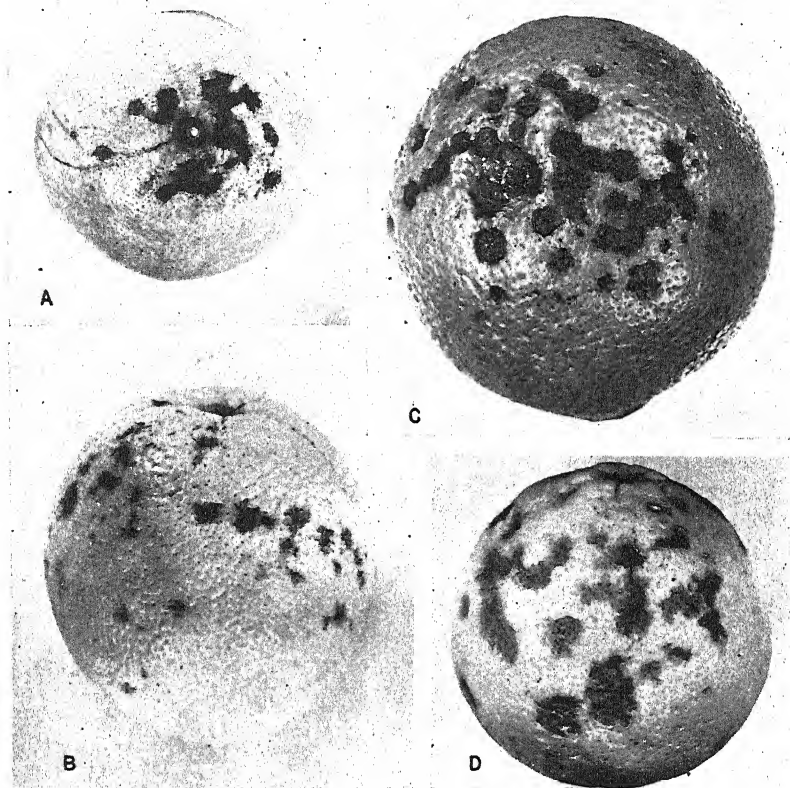
#### BROWN SPOT

In general, brown-spot lesions on navel oranges do not, in the final stages, differ greatly from those of storage spot. Usually the lesions of brown spot are shallower, more uniformly circular in outline, deeper in color, and limited to thin-skinned navel oranges. Brown-spot and storage-spot lesions have been produced on the same orange under the same experimental conditions. (Pl. 3, C.) Unquestionably, storage spot has frequently been confused with brown spot, and the greater susceptibility of those navels with smooth thin skins to both of these diseases has made distinction difficult. In general, the

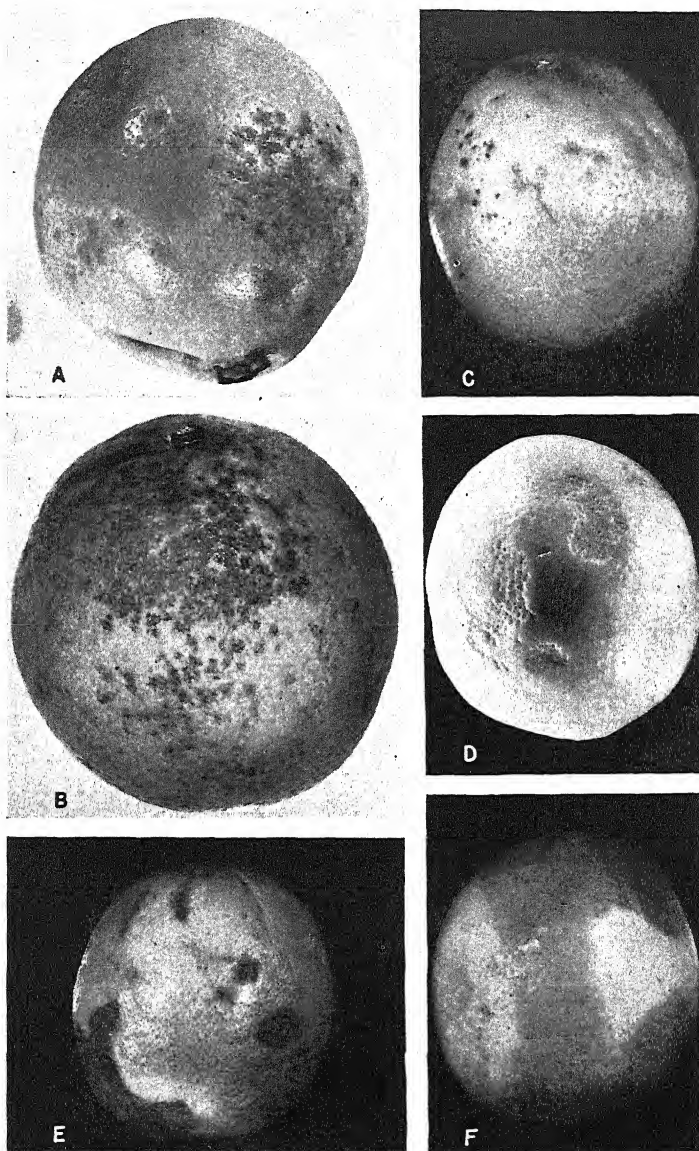
<sup>5</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.



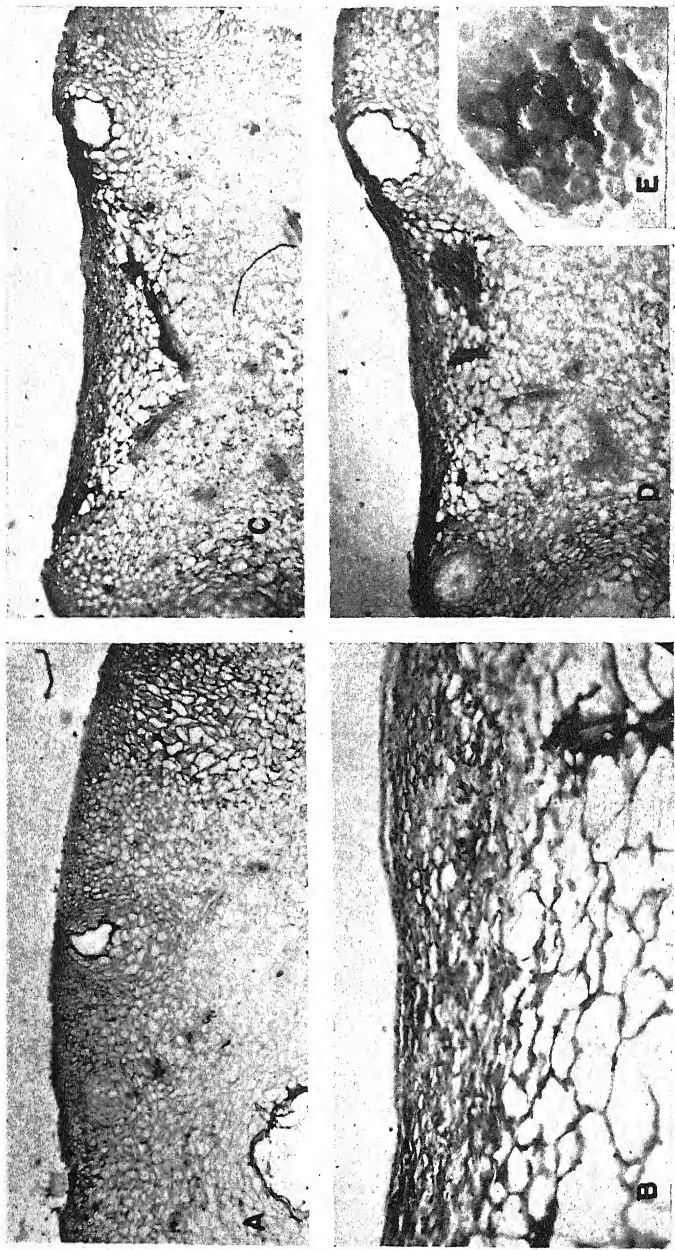
A, Florida grapefruit affected with storage spot which developed in transit. Collected March 30, 1920. B, Florida grapefruit showing a severe form of storage spot which developed in transit. Collected at Detroit terminal December 12, 1920. C, Florida grapefruit affected with storage spot which developed in cold storage. Collected July 1931. The borders of the lesions are not so dark in color as they usually are on fruit affected earlier in the season. D, Texas-grown, Marsh Seedless grapefruit affected with storage spot. Collected March 2, 1930.



A, Washington navel orange affected with brown spot at the stem end. Collected at Detroit, 1919. B, Washington navel orange affected with brown spot. Collected at Detroit, March 1920. C, Washington navel orange affected with storage spot. Specimen from a late shipment, June 1, 1926. Sometimes very ripe and old oranges and grapefruit are severely affected with this disease but usually immature fruit is more susceptible. D, Valencia orange showing the effect of storage spot. November 2, 1928.



A, Storage-spot lesions produced on Washington navel orange by storage in nitrogen for 13 days at room temperature. Thirty-minute aerations with nitrogen were given on 6 successive days. April 2, 1920. B, Washington navel orange held in open basket in cold storage for 109 days at a temperature of  $34^{\circ}$ - $36^{\circ}$  F. The fruit was green skinned when stored but gradually assumed a yellow color in storage and at the time of removal, April 23, 1929, was normal in appearance. Typical lesions of storage spot developed after 2 days at room temperature. C, Washington navel orange stored in nitrogen at room temperature for 7 days. Brown-spot and storage-spot lesions developed after 3 days in air. D, Break-down produced on Washington navel orange by storing in oxygen-free air for 4 days. The oxygen was removed from the storage jar with chemicals. March 1920. E, Lesions resembling those of brown spot produced on Washington navel orange by a short exposure to the vapor of acetaldehyde. January, 1932. F, Brown stain on Washington navel orange produced by exposing the fruit to the dilute vapor of citral. Similar but less pronounced injury follows exposure to vapors of various acetates.



A, Section through the normal peel tissue of grapefruit.  $\times 80$ . B, Section through a storage-spot lesion on grapefruit. The cells have collapsed and form a compressed layer of disorganized tissue.  $\times 80$ . C, D, Sections through storage-spot lesions on grapefruit. The collapse of the parenchyma cells and the darker colored border of the lesion is evident.  $\times 25$ . E, Surface view of a storage-spot lesion on grapefruit in an early stage of development. The cells surrounding the oil vesicles are affected, but no collapse of the oil glands has yet occurred.  $\times 8$ .

storage-spot disease is distinguished from brown spot by the more angular outline, deeper penetration into the peel, and lighter color of the lesions. Since, however, the color changes depend on the season and other variable factors, no distinctive color tones can be assigned to the lesions of either disease.

The brown-spot lesions, like those of storage spot, occur most frequently at the stem end of the fruit. This is particularly true of fruit shipped after the period of greatest susceptibility. Sometimes a few small lesions at the stem end represent the extreme development of the disease in a season. In other years the disease develops much more seriously and the fruit is badly disfigured by numerous spots over the surface.

As pointed out by Coit (2), the color of the lesions varies with the season. In some years the spots are dark brown to black and in others they are much lighter. The writer has found that as the season advances the spots become lighter in color, but occasionally fruit is observed in January or later on which the spots are almost black at the stem end. (Pl. 2, A.) The deeper color of the lesions of brown spot on fruit grown in the interior valleys and the lighter color on fruit from the coastal regions, reveals the extreme variability that may be found in the color of the spots on oranges after they reach the markets.

#### BROWN STAIN

Brown stain is a surface blemish of oranges occurring on fruit in storage, occasionally being evident also in shipments that have been long delayed. It is an important disease at low temperatures in marine shipments of oranges which are in transit for more than three weeks. It has been troublesome on export fruit from South Africa and has been observed on oranges held in storage for several weeks at temperatures below 40° F. The disease is manifest as a brown staining of the skin or flavedo, and in the beginning is unaccompanied by any depression of the surface cells. The color of the affected area is usually dark brown, closely approximating the Natal brown or cinnamon brown of the brown-spot lesions. This blemish is probably closely related to adustiosis or red-blotch disease of lemon and shows many similarities to apple scald. In the later stages, after the fruit has been exposed to drying for some time, the affected tissue is leathery and shriveled and the oil glands are slightly sunken below the level of the surrounding cells. Except in severe cases, there is no marked depression or pitting such as characterizes the lesions of brown spot and storage spot.

#### EXPERIMENTS

##### EFFECT OF OXYGEN

The experimental work on the artificial production of the diseases that have been described was begun in 1919. The first experiments were planned in conjunction with work being done on some related diseases of cabbage and other vegetables. The conditions under which the citrus diseases developed in transit and storage and the success that had been attained in reproducing the vegetable diseases in the laboratory, led to the inclusion of some experiments with oranges and grapefruit under conditions which had been effective in producing black-leaf speck of cabbage.



The first experiments consisted in storing oranges and grapefruit in large, air-tight jars with suitable connections so that aerations could be given with nitrogen or other gases. Breakdown as sunken colorless lesions, later turning brown developed on both grapefruit and oranges stored for four days in nitrogen with the gas content of the storage jars changed every 24 hours. With some variations the first experiments were repeated a number of times with similar results. In cases in which the oxygen was removed from the storage jars with pyrogallic acid and sodium hydroxide, fruits left in the jars for seven days or more developed spots in the peel similar to those observed on fruits in the market. On oranges the lesions were very similar to those of brown spot and storage spot, while on grapefruit they resembled the spots on blemished fruit from refrigerator cars and cold storage.

Experiments devised to determine the conditions under which breakdown could be produced were resumed in December, 1920. On December 8, fully colored grapefruit, Valencia oranges, California navel oranges, and lemons were stored in large, closed glass chambers of 12-liter capacity. Outlets to the storage jars were connected with tanks of nitrogen and the air in the jars was displaced with this gas. Aerations of 30 minutes duration were given daily with nitrogen, thus effecting a complete change of the gas in the jar. The oranges were stored at room temperature and also in the refrigerator at approximately 50° F. After seven days, all of the jars were opened and the fruit was held at room temperature for three days. The results of this test, which are similar to those obtained in other tests are given in Table 1.

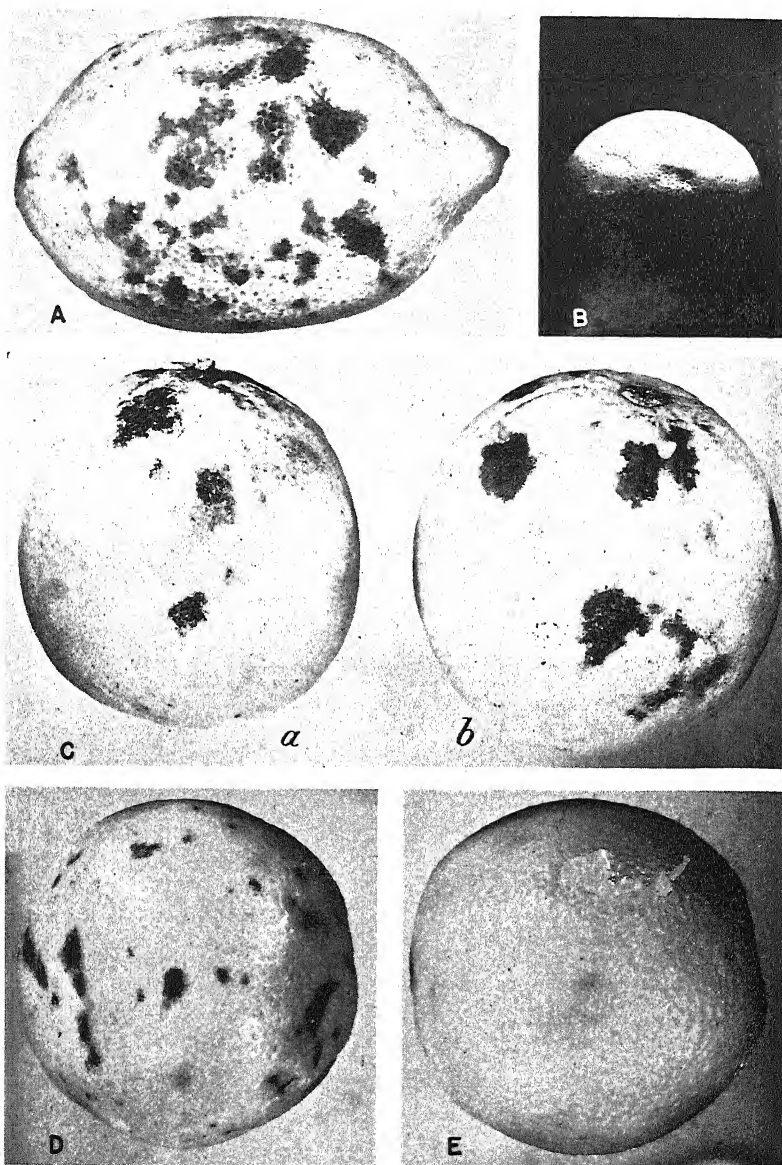
TABLE 1.—*Effect on citrus fruits of storage in nitrogen for seven days at different temperatures*

[Fruit kept in air-tight jars; nitrogen changed daily]

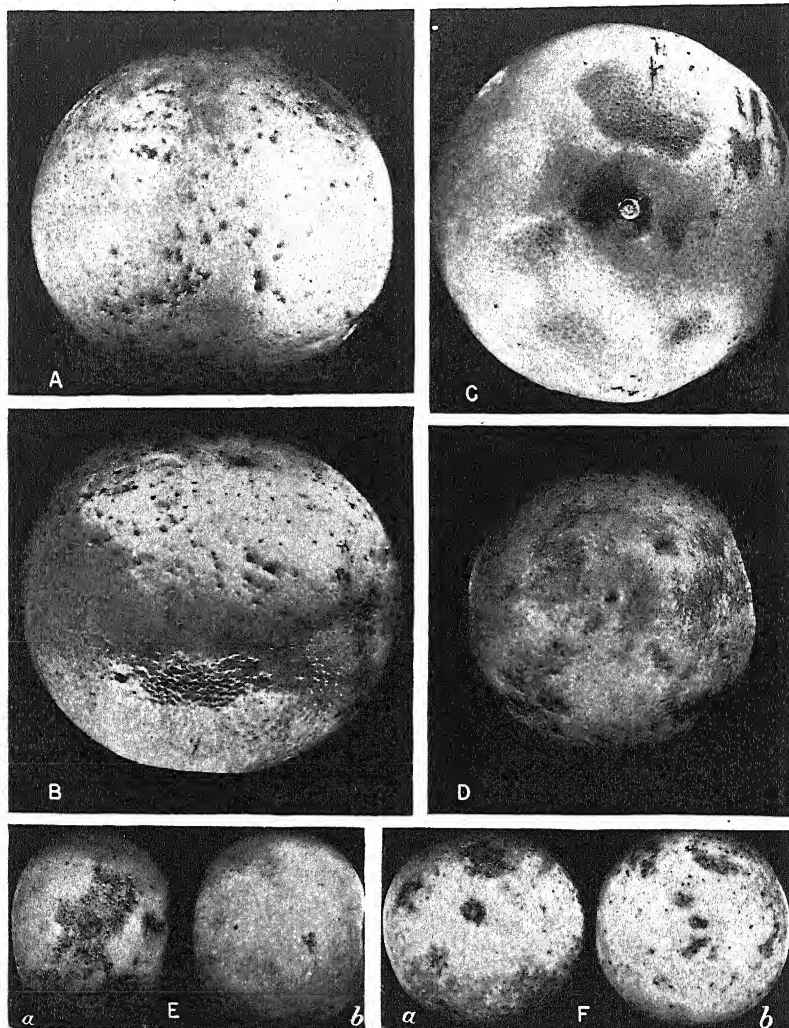
Lot No. and kind of fruit	Temperature	Results
1, oranges.....	70	Slight pitting evident on fruits before removal from jars; when exposed to air all fruits developed numerous sunken spots which gradually became Natal-brown in color.
2, oranges.....	50	Fruits developed sunken brown spots on exposure to air; spots 1-2 cm in diameter, irregular border, Natal-brown color.
3, grapefruit.....	70	Pitting visible on the fruits after 3 days in jars; very numerous after 7 days; small pits involving only small area around oil glands; border of the spots irregular; spots more numerous at stem end; hydrangea-red color after 3 days at room temperature.
4, lemons.....	70	All fruits developed deeply depressed spots in the peel; 1-2 cm in diameter, colorless at first, pale vinaceous after 3 days.

When the oranges were removed from the storage jars only a few lesions were visible in the peel, but after exposure to air, pitted areas developed over the surface. The lesions were colorless at first but within 24 hours the affected areas were cinnamon brown. (Pl. 5, C, a.) The spots were sharply sunken and the oil glands had collapsed also. On some fruits, brown scalded areas were evident. The scalded tissue gradually dried out and the affected areas finally appeared on the peel as slightly depressed, Natal brown spots. Small sunken spots were visible on the grapefruit after three days in the storage jar, and after seven days the number and size of the lesions had greatly in-





A, Lesions of the storage-spot disease on lemons collected December 3, 1923. B, Breakdown in the form of sunken lesions produced on lemons by storage in nitrogen atmosphere for 5 days at room temperature. C, Brown spot of Washington navel orange (*b*) on fruit collected January 4, 1920, and similar lesions produced on thin-skinned navel orange (*a*) by storing in nitrogen for 7 days. The gas in the storage jar was changed daily by a 30-minute aeration with pure nitrogen. D, Brown-spot lesions occurring on Washington navel orange after storage in nitrogen for 5 days. December 4, 1920. E, Washington navel orange from the check of the same experiment (D) stored in air-tight jar for 5 days and given a daily aeration of 30 minutes with air of normal composition.



A. Storage-spot lesions developed on green-skinned Florida grapefruit after storage for 7 days at 40° F. B. Storage-spot lesions on green-skinned Florida grapefruit stored 10 days in air constantly circulated but not renewed. (See Table 3.) The lesions developed before and after removal from the storage jar. Experiment begun February 5, 1921. C. Large lesions on green-skinned Florida grapefruit stored in air-tight jar for 10 days. Aerations of 30 minutes with nitrogen were given each day. D. Sunken lesions, which gradually developed a vinaceous color, produced on grapefruit by exposure to the dilute vapor of acetaldehyde and acetic acid. E. Texas grapefruit that has been wrapped in commercial (a) and oiled (b) wrappers and stored for 6 weeks at 34°-36° F. Storage spot is severe on a, but b is almost free from spotting. F. Texas grapefruit that had been wrapped in commercial (a) and oiled (b) wrappers and stored for 7 weeks at 34°-36° F. The fruit from both the oiled and commercial wrappers spotted badly. The same results were obtained with fruits from the same shipment stored under identical conditions for 10 weeks.

creased. The spots were formed by the collapse of the cells surrounding the oil glands. Later the glands also were involved. The lesions were not colored when the fruit was taken from the jars but 48 hours later they were hydrangea red. The spots varied from 2 mm to 4 cm in diameter, and were mostly circular in outline.

The lemons were affected with deeply sunken, colorless lesions before they were removed from the storage jar. When the jar was opened a strong odor of lemon oil was detected. The spots in the peel were 1 to 2 cm in diameter and resembled the lesions of the peteca disease. (Pl. 5, B.) They were colorless when the fruits were taken from the jars but after 3 days the affected tissue was pale vinaceous.

This experiment is typical of others in which early-season ripe fruit was used. In practically all cases, breakdown in the form of sunken lesions of variable size has followed storage of the fruit in nitrogen for 5 to 10 days. Oranges or grapefruit that are still green in color are more susceptible to injury than yellow fruit. The reaction of green grapefruit to similar storage conditions was tested with some market fruit shipped in October, 1921, from the Manatee River district of Florida. The fruit was stored on October 21 in large glass jars. A stream of nitrogen was blown constantly over the fruits in some of the jars; in others the gas was not changed. The result was the same, breakdown occurring in the form of sunken colorless spots in the peel after three days of storage at room temperature. This represents about the minimum time necessary to produce breakdown by storing fruit in the most susceptible condition in nitrogen. A much longer period of storage is necessary to produce spotting on yellow fruit, and in some cases breakdown has not occurred after storage in pure nitrogen for seven days.

In the preliminary tests with oranges, similar results had followed storage in nitrogen and in air from which the oxygen had been absorbed. The results of a typical experiment with navel oranges stored in nitrogen are shown in Table 2.

TABLE 2.—*Effect of various storage conditions in producing surface breakdown of navel oranges in tests at room temperatures, March 25, 1920*

Lot No.	Method of storing	Storage period	Results
		<i>Days</i>	
1	Oxygen absorbed with pyrogallie acid and KOH; aerated 30 minutes daily with nitrogen.	4	All oranges developed sunken, colorless spots on exposure to air; spots numerous, variable in size; pecan-brown color after 3 days.
2	Same as 1, but aerated 1-hour daily with nitrogen.	7	Sunken, colorless spots visible in the peel after 5 days; when removed from the jars all oranges developed numerous sunken colorless spots over the surface; orange-cinnamon color after 3 days.
3	Oxygen absorbed with pyrogallie acid and KOH; no aeration with nitrogen.	4	When removed from the storage jars, all oranges developed numerous large sunken lesions, formed at first by the collapse of the cells around the oil glands; pecan-brown color after 3 days.
4	Stored in pure nitrogen; gas not changed.	4	Lesions similar to those on the fruits from jar 3; 1-2 cm in diameter; pecan-brown color.
5	Stored in pure nitrogen; aerated 1 hour daily with nitrogen.	13	Fruits severely spotted with large, sunken lesions similar to those on fruits in jars 3 and 4.
6	Stored in air-tight jar; aerated 1 hour daily with air of normal composition.	13	All fruits sound.
7	Wrapped with commercial wrappers and held in open basket.	13	Do.

The reaction of oranges to storage in nitrogen or in air from which the oxygen had been absorbed was not different from that of grapefruit under similar conditions. In the jars aerated for one hour daily with nitrogen, breakdown was as severe as in others in which the gas was not changed. Tests with navel oranges in January, 1932, gave similar results. Thin-skinned navel oranges were stored in two large glass jars and the air in one was displaced with nitrogen. The fruit in the second jar was exposed to a constant stream of nitrogen, which resulted in a complete change of the gas in the jar every hour. At the end of seven days the oranges were removed from the two jars and held at room temperature for three days. No breakdown was visible at the termination of the storage period on any of the fruits which had been exposed to a constant stream of nitrogen, but typical pox lesions developed within three days. Of the fruits from the jar in which the nitrogen was not changed, characteristic brown-spot lesions were evident on one when the jar was opened. At the end of three days typical storage-spot lesions had appeared on all the fruits.

The results of this and similar experiments indicate that breakdown occurs when oranges are stored for a short time in air freed of oxygen. The period of exposure required to cause injury depends on the condition of the fruit. Some tests have been conducted with green-skinned navel oranges shipped directly to the writer from the grove in California. Fruit of this kind breaks down much more rapidly under the storage conditions that have been described than ripe oranges. These results are in accord with many observations which have shown that fruit in a slightly green condition is much more susceptible to spotting in transit than ripe fruit.

#### EFFECT OF AIR MOVEMENT

In the experiment with oranges and grapefruit stored in nitrogen, breakdown was not prevented by blowing a constant stream of gas over the fruit. Brooks, Cooley, and Fisher (1) have demonstrated the beneficial effect of air movement in dissipating the volatile products of respiration which injure the superficial layers of the cells of apples in cold storage. Since apple scald has been well controlled by aeration, and also by the use of wrappers which absorb these toxic substances, the importance of determining whether or not the citrus breakdown was due to similar causes was early recognized. Some experiments on air movement and the use of wrappers impregnated with absorbents effective in the control of apple scald were devised to determine, if possible, the nature of the citrus diseases.

The first experiment on the effect of air movement was carried out in September, 1921, with green Isle of Pines grapefruit. The fruit was stored in 14-liter jars, and an apparatus illustrated in a previous publication (13) was used to circulate the air in the storage chamber. The experiment was conducted at room temperature but, owing to the warming of the air by the heat from the motor-driven fan, the temperature of the circulating air was approximately 77° F. The fruit was left in one jar for 7 days and in the second for 13 days. When removed from the first jar at the end of seven days the fruits were perfectly normal in appearance, yellow, and free from blemishes. After three to five days, small, sunken spots, beginning as a collapse of the cells surrounding the oil vesicles, appeared on all the fruits.

The spots were 1 to 5 mm in diameter, and although colorless when first exposed to the air, they became cinnamon brown within three days. There was no off-taste or impairment of the eating qualities of the fruit as a result of the storage conditions.

The second jar was opened at the end of 13 days; at this time some of the fruits were already spotted but 3 days later the number and size of the lesions had greatly increased. This fruit also compared favorably in flavor with the check fruit ripened in the air of the laboratory. The development of the spots and color changes were similar to those on the grapefruit stored for only seven days.

A second experiment of a similar nature was started on February 5, 1921, with slightly green grapefruit. The air was circulated around the fruit but not renewed. In a second jar the fruit was stored in nitrogen and the gas changed every 24 hours by a 30-minute aeration. The results of this experiment are shown in Table 3.

TABLE 3.—*Effect of composition and movement of storage air on pitting of grapefruit in tests at room temperature during a 10-day storage period*

Lot No.	Method of storing	Results
1	Air stirred constantly but not renewed.....	Pitting in the peel evident after 7 days. After removal from jars all fruits developed few to many spots; spots hydrangea red after 3 days; fruit normal in flavor and color.
2	Stored in nitrogen and aerated 30 minutes daily with this gas.	Fruits severely disfigured by sunken areas in the peel; evident after 5 days but developed more severely after the fruit was removed from the storage jar; hydrangea red after 3 days.
3	In open basket.....	All fruits sound, slightly shriveled, yellow.

Lesions very typical of storage spot appeared on the fruits from the jars in which the air was circulated but not renewed. The spots were generally small, but on some fruits large areas were involved by the collapse of the cells surrounding the oil vesicles. (Pl. 6, B.) The lesions on the fruits stored in nitrogen were much larger, consisting of sunken areas which included the oil glands as well as the adjacent cells. These lesions were visible on the fruits before they were removed from the storage jar but were colorless until exposure to the air of the laboratory caused color changes similar to those previously described.

Although in these experiments a continuous circulation of the air in the storage jars did not prevent the development of breakdown, it is not to be concluded that under conditions more nearly like those prevailing in storage and transportation, air movement would not have a beneficial effect. In these jars the temperature was much higher and the relative volume of fruit per unit volume of air much in excess of that to which citrus fruits are usually exposed in storage and transportation.

#### EFFECT OF TEMPERATURE

In the preceding experiments all of the tests except one were carried out at room temperature. To determine the effects of low temperature on grapefruit, a case of green Florida fruit was purchased in November, 1925, and stored at various temperatures. In general, all the fruit used was green-mature but some variation in color was unavoidable. The storage conditions and temperatures employed are indicated in Table 4.

TABLE 4.—*Effect of temperature on the development of storage spot or pox on green-skinned grapefruit stored by different methods for varying periods*

Lot No.	Method of storing	Temperature	Storage period	Results
1	In wooden box with closely fitting lid.	° F. 31-33	Days 22	A few fruits pitted at time of removal from storage; within 2 days after removal from storage every fruit affected with typical storage spot.
2	In 12-liter, air-tight glass jar.....	40	17	All fruits affected with typical storage-spot disease within 2 days after removal from storage.
3	In open basket.....	31-33	22	Fruits badly spotted within 24 hours after removal from storage.
4	do.....	40	17	Do.
5	do.....	70	17	All fruits sound.
6	do.....	50	42	Do.

As Table 4 shows, the fruits stored in open baskets or in closed jars spotted badly at the lower temperature. There was no deficiency of oxygen in the air of the storage rooms at any of the temperatures employed. In the 31°–33° F. room absence of other living material precluded the possibility of the oxygen content of the air being reduced. In addition, the doors of this room were opened at frequent intervals during the day for the removal of dairy products. Despite the abundant supply of oxygen in the air, the fruits in open baskets spotted as badly as those in the closed containers. Since the grapefruit in the open baskets were not wrapped, free movement of air around each fruit was not prevented. At both 50° and 70° no spotting developed on any of the fruits.

Although the results of the experiments on air movement, temperature, and aeration with nitrogen indicate that spotting of grapefruit is not produced by any volatile substance acting at the surface of the fruit, there is not excluded the possibility that a slow, cumulative adsorption of such substances at low temperatures may cause this type of injury. Whatever the cause of the injury may be, the tolerance of green fruit to cold is very low compared to that of apples. Spotting occurred in one case on green fruits after only seven days storage at 40° F. (Pl. 6, A.)

#### INFLUENCE OF MATURITY

In some of the experiments described it was noted that green fruit was much more susceptible to injury at both high and low temperatures than ripe, yellow fruit. Coit (2) noted the greater susceptibility of early picked oranges to brown spot, and Hawkins and Magness (9) observed that grapefruit were most susceptible to breakdown in cold storage immediately after picking. To compare the resistance of green and ripe fruit some tests were made in November, 1925, with Florida grapefruit. Two cases of grapefruit from the same shipment were obtained. One lot was green in color and the other had the normal yellow color of ripe fruit. Uniformly green and fully colored fruits were selected and stored as indicated in Table 5.

TABLE 5.—*Influence of maturity on the susceptibility of grapefruit to breakdown at various temperatures when stored for different periods of time*

Color of fruit	Method of storing	Temperature	Storage period	Results
Green.....	Tightly closed wooden box.....	° F. 31-33	Days 22	All fruits spotted after 48 hours at room temperature; an occasional fruit was already spotted when removed from storage.
Do.....	Air-tight glass jar.....	33-42	17	Spotted after 3 days at room temperature.
Do.....	Open basket.....	31-33	22	All fruits spotted within 24 hours at room temperature.
Do.....	do.....	33-42	17	Spotted after 2 days at room temperature.
Do.....	do.....	70	17	All fruits sound, yellow in color, and slightly shriveled.
Yellow.....	do.....	31-33	28	All fruits sound.
Do.....	do.....	33-42	17	Do.
Do.....	do.....	31-33	42	Do.
Do.....	do.....	43-52	42	Do.

The maximum period of storage for the green fruit was 22 days, but one lot of the yellow fruit was left for 42 days at the lowest temperature. In all cases green fruit stored at 31°-33° and 38°-42° F. became spotted before or shortly after removal from storage. At the lower temperature some of the fruit spotted while still in storage, but most of the spotting occurred after exposure to the warm air of the laboratory. At the end of 42 days, the yellow fruit stored at 31°-33° was still sound and remained so after exposure to warm conditions.

In experiments with other lots of grapefruit, results similar to these were obtained. Fruit that is mature and fully colored is comparatively resistant to storage spot. This is more especially true of mid-season shipments. Fruit that is fairly well colored but picked early in the season seems to be more susceptible than similarly colored fruit picked later. According to legal standards, grapefruit and oranges with green skins may be shipped if they contain sufficient sugar and the acid content is low enough. In these shipments, which often arrive at destination in a green condition, breakdown is frequently very severe. Both grapefruit and oranges of midseason and late shipments are ordinarily fully colored and mature and usually are not seriously affected with storage spot.

Some exceptions have been observed where storage spot has developed severely on ripe and very old fruit. Similar exceptions to the rule that immature apples are more susceptible to apple scald have been noted by Brooks, Cooley, and Fisher (1).

#### EFFECT OF OILED AND WAXED WRAPPERS

The beneficial effect of oiled wrappers in the control of scald on apples in cold storage suggested the possibility that the use of these might prevent the spotting of citrus fruits in transportation and storage. The first wrappers tested were prepared by treating the ordinary commercial wrappers with olive oil, beeswax, and cocoa butter according to the method used by Brooks, Cooley, and Fisher (1). In a test conducted in 1920, grapefruit and oranges wrapped in the prepared wrappers and stored in nitrogen at room temperature for 12 days spotted badly when exposed to the air.



In 1924 the writer obtained some commercially prepared oiled wrappers through the courtesy of D. F. Fisher of Wenatchee, Wash. These wrappers carried about 12 per cent of mineral oil and were effective in preventing apple scald. An early shipment of Indian River grapefruit obtained October 14, 1924, was used for the first test. The fruit was slightly green and had a smooth, fine-textured skin. Since no cold-storage facilities were available, the fruit was stored only at room and ice-box temperatures. The results of this test are shown in Table 6.

TABLE 6.—*Effect of oiled wrappers on the spotting of grapefruit, when stored for different periods of time*

Lot No.	Method of storing	Temperature	Storage period	Results
		° F.	Days	
1	Wrapped in oiled wrappers; stored in air-tight jars.	70	10	All fruits spotted 2 days after exposure to air at room temperature; spots small and few in number.
2	Wrapped in commercial wrappers; stored as in 1.	70	7	All fruits slightly spotted after 3 days at room temperature.
3	Unwrapped; in open basket.	70	10	All fruits sound.
4	Wrapped in oiled wrappers; in air-tight jar.	50	8	Do.
5	Wrapped in oiled wrappers; fruit stored in open baskets.	50	10	Do.

At room temperature the oiled wrappers were ineffective in preventing spotting of the fruits in closed jars. Analyses made every 24 hours of the gas content of the jars kept at room temperature showed that the oxygen was rapidly depleted. After 64 hours only 1.5 per cent oxygen remained in the jars, and after 108 hours no test for this gas could be obtained by the method used (Ganong (5)). At the lower temperature no breakdown developed on any of the fruits, either in the air-tight jars or in open baskets. The storage period was too short at this temperature to cause breakdown, although in some earlier tests, green-skinned fruits had become spotted in seven days at temperatures slightly above 32° F.

Florida grapefruit wrapped in commercial and oiled wrappers and stored for six weeks, beginning January 13, 1932, at temperatures of 34°–38° F. became slightly spotted after removal from storage. None of the fruits in either kind of wrapper was seriously spotted. On March 1, 1932, a second experiment with the oiled wrappers was started in which mature, fully colored, Texas-grown Marsh Seedless grapefruit were used. One lot was stored at 34°–36° for 6 weeks; a second lot was stored at 34°–38° for 7 weeks, and a third lot was stored at 34°–38° F. for 10 weeks. Fruits wrapped with the ordinary unoled commercial wrappers were stored under the same conditions. The oiled wrappers reduced the amount of spotting on the fruits stored for 6 weeks at 34°–36° (pl. 6, E), but those stored for 7 weeks at 34°–38° F. spotted as badly in the oiled as in the commercial wrappers (pl. 6, F). The oiled wrappers had no apparent beneficial effect on the fruits held for 10 weeks at the same temperature.

In December, 1925, navel oranges wrapped in the commercial unoled wrappers and the same brand of oiled wrappers that were used in the grapefruit experiments were stored for 45 days at 34°–38° F. The first spotted fruits were found in the oiled wrappers after 34 days



of storage; after 45 days all the fruits were removed from storage, unwrapped, and held for 3 days at room temperature. None of the oranges had spotted badly, but those from the oiled wrappers were in no better condition than those from the commercial wrappers.

In a similar experiment with navel oranges wrapped in both commercial unoled and oiled wrappers and stored January 13, 1932, at 34°–38° F. for 47 days slight spotting developed on all the fruits from both the oiled and the unoled wrappers. There was slightly more spotting on the fruits from the unoled wrappers, but the difference did not appear to be significant.

The favorable influence of the oiled wrappers in some of the grapefruit experiments warrants further trials of this method, with wrappers of higher oil content and fruit freshly picked from the trees. It is possible that these wrappers might delay the appearance of the disease in early shipments of grapefruit and oranges. Friend and Bach (4) have recently reported beneficial results from waxed wrappers on Texas grapefruit in cold storage.

#### EFFECT OF CHEMICALS

The discovery by Brooks and his associates that certain volatile emanations of apples are the probable cause of scald in storage marked a milestone of progress in the search for methods of controlling this very serious disease. The results obtained in the experiments conducted to produce scald under laboratory conditions influenced the writer to test various chemicals for their effect on citrus fruits. These experiments, like the preceding, were conducted with fruit that was not especially favorable for experimentation. It is believed that the results would have been more decisive had the fruit been in the most susceptible condition.

Recent investigations by Klotz (10), of red blotch, and peteca of lemon, diseases that develop in the curing house where ventilation is poor, have shown that these blemishes are probably caused by the action of toxic substances produced in the respiratory processes of the fruit. Touching the fruits with a number of esters or injecting small amounts of citrus oils under the flavedo produced lesions typical of these two diseases.

Various substances besides a number of aldehydes, esters, and alcohols that are concerned in the chemistry of citrus, were used in attempts to simulate the effects of brown stain, brown spot, and storage spot on oranges and grapefruit. The chemicals tested included acetaldehyde, acetic acid, citral, citronellal, geraniol, methyl anthranilate, linalool, *d*-limonene, ethyl acetate, methyl acetate, amyl acetate, and octyl acetate. The method used was to expose the fruit in closed vessels to the chemical in a vaporized condition. One to two milliliters of the chemical to be tested was poured in the bottom of an 8 to 12 liter desiccator and the fruit was placed on supports a few inches above the concentrated material. The desiccators were kept at room temperature or in the refrigerator at 40° F. The period of exposure varied from 2 to 24 hours, depending on the condition of the fruit, the temperature, and the concentration of the chemical.

The experiments were repeated a number of times, and so far as possible with fruit in different degrees of susceptibility. The results have been fairly consistent, with no extremes of variation, except in

degree of injury due to period of exposure, concentration of chemical, or condition of the fruit used. Green fruit has shown the greatest sensitiveness to injury from the chemicals tested, and there is apparently a progressive decrease in this sensitiveness as the season advances and the fruit is more mature when shipped.

Injury to oranges, closely resembling the brown-stain disease, was produced by exposing fruit to the vapors of citral, citronellal, and various acetates, including ethyl, amyl, methyl, and octyl. (Pl. 3, F.) With a concentration of 1 to 2 ml of citral in an 8 to 10 liter desiccator, typical surface injury resembling the brown-stain disease was produced in a few hours. Longer exposures at 40° F. produced a similar effect, and the injury produced with low concentrations of citral and long exposure was indistinguishable from the stain on oranges resulting from cold storage. The acetates also were effective, but oranges were more resistant to their vapors than to those of citral.

Geraniol vapor caused no injury either to oranges or to grapefruit at low and moderate concentrations. Oranges were exposed for several days without apparent injury to the vapor of this alcohol at the concentration at which citral caused marked injury within a few hours.

Methyl anthranilate caused injury to oranges when the fruit was exposed to the concentrated vapor of the chemical for longer periods of time. With the concentration of citral doubled, three to five days were required to cause visible injury in the form of sunken and scalded areas in the peel. Linalool and *d*-limonene caused no injury to oranges or grapefruit at similar concentrations.

In both oranges and grapefruit, sunken lesions in the peel followed exposure to the vapor of acetaldehyde. These lesions were more nearly like those that characterize injury from low temperature than those produced by other chemicals. (Pl. 6, D.) Usually the lesions of typical storage spot originate in a collapse of the cells surrounding the oil vesicles. This is not invariably the case, however, for sometimes the oil glands show the initial effects of the disease. Some lesions produced with acetaldehyde began with the collapse of the cells above the oil vesicles, which was followed later by the collapse of the cells between the vesicles. Where only slight injury followed exposure of the fruit to the dilute vapor of acetaldehyde the effect was noticed only in the tissue directly above the glands.

When low concentrations of acetaldehyde were used the lesions formed on navel oranges resembled very closely those of the brown-spot disease. (Pl. 3, E.) When greater concentrations were used the entire surface of the fruit became scalded and pitted. With slightly green and very susceptible fruit the type of injury could be made to simulate brown spot or storage spot by varying the concentration and the period of exposure. Yellow ripe fruit that had been picked for several weeks was comparatively resistant to injury by this chemical. Fruits with smooth, fine-textured skin were most susceptible, but typical lesions were also produced on coarse thick-skinned fruits.

Lesions resembling those produced with acetaldehyde have developed also on fruit exposed to the vapor of acetic acid. One to two ml of 50 per cent acetic acid in an 8-liter desiccator affected oranges and grapefruit in very much the same way as did acetaldehyde. A mixture of acetaldehyde and acetic acid was more effective than either alone. In some of the earlier experiments, acetaldehyde that had

been stored for some time at room temperature was used, and fruit exposed to this lot was much more quickly injured than other lots exposed to acetaldehyde that had recently been purchased. Upon investigation it was found that the acetaldehyde had undergone oxidation and acetic acid had been formed in considerable quantity.

The color reactions of the tissues injured with acetaldehyde are very nearly the same as those that occur in the development of brown-spot and storage-spot lesions. The affected tissue is at first colorless, but, from a pale vinaceous shade, progressive color changes take place which proceed finally to the tones that characterize the natural lesions of these diseases. The purplish tinge so characteristic of the border portion of the brown-spot lesion on naval orange is especially noticeable around the margin of the injured tissue that develops after exposure to the vapor of acetaldehyde. On grapefruit the darker border of the lesion produced by acetaldehyde is very similar to that of the natural lesion.

### DISCUSSION

In a preceding paper the writer (13) described certain functional diseases affecting vegetables in storage and transportation and showed by experiments that these diseases could be produced by exposing susceptible vegetables like potato, cabbage, and lettuce to conditions in which the quantity or the availability of oxygen was reduced. Other investigators had previously shown the liability of plants to injury at temperatures above the freezing point and had concluded that the injury was due to the accumulation of toxic substances as a result of incomplete cellular oxidations. Though the ultimate cause of the breakdown of the vegetables at low temperatures and under conditions of reduced oxygen tension was not determined, it was suggested that suboxidation (referring to respiration reactions) was of primary importance as a precursor of the toxicity actually causing the death of the cell. The occurrence of the blemish diseases of citrus fruits under similar conditions in storage and transportation, and the comparable reaction of these fruits to the conditions imposed in the vegetable experiments, show the qualitative relation of these blemishes and suggest again the rôle of suboxidation in their production. The circumstances that bring about reduced availability of oxygen in susceptible cells may be storage conditions of poor ventilation or low temperatures which prevent the utilization of oxygen requisite for normal respiration. The diseases of citrus fruits described as brown stain, brown spot, and storage spot thus appear to be manifestations of suboxidation reactions in the most active cells of the peel. It should be emphasized that the ultimate cause of each of these diseases is not yet known, although the results of some of the tests with chemicals may afford suggestive evidence.

Brown stain of orange is very similar in appearance to apple scald, and the experimental production of this disease with substances which produce scaldlike effects upon apple reveals the close similarity of the two disorders. The most typical injury was produced with the vapor of citral. The brown staining of the surface of orange which followed a short exposure to the vaporized substance was indistinguishable from the disease as it occurs naturally on oranges from cold storage.

The results of the experiment on air movement and the influence of temperature and of oiled wrappers seem to indicate that storage

spot and brown spot are probably not due to the surface action of poisons that can be dissipated by air currents or absorbed by neutral oils. The results of the oiled-wrapper experiments are, however, not conclusive enough to warrant an unqualified statement.

The manner in which the lesions of storage spot develop indicates that the initial site of injury is the deeper-situated parenchyma cells that circumscribe the oil vesicles. In the great majority of cases the cells over the oil glands do not, in the beginning, appear to be injured, and the breaking down of the peel tissues seems to originate in the subsurface parenchyma cells. Later, through desiccation of the cells surrounding the oil vesicles, the glands and the overlying cells are also affected. Such a sequence of events suggests that the initial injury occurs in deeper-situated cells and progresses outward.

The progressive increase in the permeability of the cells of the endocarp of orange at low temperatures indicates that such temperatures are injurious to the cell membranes. The most plausible explanation for this increase in permeability is the accumulation of poisonous substances within the cell. Since Pantanelli (14) demonstrated that at low temperatures an autodigestion of the proteins of orange occurs, it is evident that there is more than a theoretical basis for this explanation. Zoller (24) has shown that naringin, the bitter principle in the peel of grapefruit, gradually disappears during storage, and he suggests that certain flavors and "pink spots" which develop simultaneously with its disappearance are to be traced to the reactions of this glucoside. The products of the hydrolysis of naringin are glucose, rhamnose, and the phloroglucinol ester of *p*-hydroxy-cinnamic acid, and conceivably a gradual accumulation of this ester at low temperatures might be concerned in the occurrence of storage spot.

The causal factors in such functional diseases of apple as scald, internal browning, deep scald, etc., is a disputed question. These diseases develop in cold storage, and the most generally accepted view has been that of Brooks and his associates, who believe that injury to apples in the form of superficial scald is brought about by the action of poisonous substances like acetaldehyde and fruit esters which accumulate at low temperatures. The beneficial effects of ventilation and of oiled wrappers in preventing this type of breakdown lend support to this view. Thomas (21) and Trout (23), however, believe that the cells first become unhealthy from some cause as yet unknown and that following the initial injury zymasis occurs and the accumulated by-products accentuate the injury. According to this view acetaldehyde does not accumulate in apples or pears until the cells are injured from some other cause.

It is not proposed to consider here the controversial aspects of this problem. In the experiments with oranges and grapefruit, injuries resembling the lesions of brown spot and storage spot were produced by exposing these fruits to the dilute vapors of acetaldehyde and mixtures of acetaldehyde and acetic acid. Acetaldehyde is believed to be an intermediate product of respiration and under anaerobic conditions its production is increased (Kostyschew, Hübner, and Scheloumoff (11) and Thomas (21)). Of interest is the fact that acetaldehyde may be formed from citral in the presence of weak alkalis. Whether or not such a reaction might occur in citrus fruits in storage is not known. Since no analyses for acetaldehyde

in diseased tissues were made it can not be stated whether it is concerned in the causation of brown spot or storage spot. The fact that lesions resembling those of brown spot and storage spot can be produced by exposing fruit to dilute vapors of acetaldehyde or certain fruit esters suggests that some substance having a similar action may be concerned in the occurrence of these diseases at low temperatures or under conditions of poor ventilation.

Two hypotheses may be proposed for the injury to citrus fruits in cold storage. Low temperatures may cause a preponderance of hydrolytic activity resulting in excessive splitting of substances like glucosides, with a resultant accumulation of materials which poison the protoplasm. This explanation is supported by the partial control of pitting by prestorage treatment of grapefruit with kerosene-stove gas. This treatment speeds up katabolic processes greatly and must cause a marked decrease in storage materials like glucosides. The effects of accumulated products of incomplete respiration offers an alternative explanation for breakdown at low temperatures. Under normal conditions poisoning of the protoplasm is prevented by oxidation of toxic materials as fast as they are produced, but at low temperatures the oxidizing system in the cell is affected to such an extent that there may be a slow accumulation of these materials and eventual injury to the protoplasm. Low temperatures are evidently much more injurious to citrus fruits than to apples, since storage for 2 to 3 weeks at 32°-34° F. results in injury to grapefruit, whereas under similar conditions apples are not affected before 8 to 12 weeks.

The practical importance of oiled wrappers in the control of functional diseases of fruits in cold storage makes it desirable that conclusive evidence be obtained as to their value with oranges and grapefruit. The tests reported in this paper both with chemicals and wrappers should be repeated with fruit in condition likely to yield decisive results. In these experiments only market fruit was available and nothing was known of the previous treatment to which it had been subjected. More decisive results would probably follow a repetition of the experiments both with chemicals and oiled wrappers by using fruit freshly picked at a period when it was most susceptible to these blemish diseases.

#### SUMMARY

This paper reports the results of studies on certain functional diseases affecting citrus fruits in storage and transportation. The investigation deals chiefly with the storage spot or pox disease, but some experiments on brown spot and brown stain of orange are included.

The symptoms of these diseases are the result of a breakdown of the peel tissues following storage of fruit under conditions of poor ventilation or where the temperature is so low that normal respiratory functions are deranged.

Storage spot and brown spot have been produced in the laboratory under various conditions of air composition, and typical symptoms have followed storage of susceptible fruit in nitrogen for 4 to 10 days.

Under the conditions of these experiments air movement without renewal has not been effective in preventing storage spot at room temperature.

Storage spot has been produced at low temperatures where there has been no deficiency of oxygen in the surrounding air. Storage of green-skinned fruit for 10 to 14 days at temperatures close to 32° F. is sometimes sufficient to cause typical breakdown. Temperatures from 32°-42° are effective in producing breakdown of oranges and grapefruit where there is no oxygen deficiency in the surrounding air.

The susceptibility of oranges and grapefruit varies with the degree of maturity. Green fruit is usually more susceptible than colored and fully matured fruit.

Oiled wrappers have given variable results in the control of storage spot and brown spot. In most cases they have not decreased the amount of disease on either grapefruit or oranges in cold storage, but in a few instances they apparently have been beneficial.

Injury closely resembling brown stain has been produced on oranges by exposing them to the dilute vapors of certain chemicals. Citral has been especially effective in causing this type of injury. Similar injury has been produced with various acetates which also produce scald-like effects on apple.

Brown stain is a disease apparently very similar to apple scald with the injury incident in the surface cells and caused by the action of certain cumulative products of the orange itself.

Oranges and grapefruit exposed to the vapors of acetaldehyde, acetic acid, or a mixture of acetic acid and acetaldehyde have developed lesions in the peel resembling those characteristic of brown spot and storage spot. Other factors being equal, the degree of injury produced depends on the maturity of the fruit.

The lesions of brown spot and storage spot appear to arise through injuries in the parenchyma cells surrounding the oil vesicles. The effect of acetaldehyde and fruit esters upon oranges and grapefruit suggest that some substance having a similar action may be concerned in the causation or accentuation of the injury that follows storage of these fruits at low temperatures.

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# EVAPORATION FROM SALT SOLUTIONS AND FROM OIL-COVERED WATER SURFACES<sup>1</sup>

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## INTRODUCTION

Evaporation experiments performed at Fort Collins, Colo. (6)<sup>2</sup>, and at various points in the western part of the United States at different altitudes, under a wide range of meteorological conditions, show that there is a definite relation between the evaporation and the difference in vapor pressure, the wind velocity, and the altitude.<sup>3</sup> There are, however, other factors, such as oil films on the water surface and the presence of various salts in the water, which are known to influence the evaporation from free-water surfaces.

The idea has been advanced that evaporation losses from reservoirs can be reduced materially by maintaining an oil film over the surface, and withdrawing the water for irrigation from below the reservoir surface without disturbing the oil film. Frequently irrigation water contains considerable quantities of salts in solution, and in experimental work it is sometimes desirable to add small amounts of salt to evaporation tanks in order to prevent the growth of algae in the water. Therefore it is important to know how the rate of evaporation from salt solutions and oil-covered water surfaces compares with that from fresh water.

In order to determine the effect of these factors on evaporation, and the influence of the meteorological factors under these conditions, experiments were performed at Fort Collins on the evaporation from oil-covered water surfaces, and from various solutions of sodium chloride and sodium sulphate. The results of these tests are reported in this paper.

## EFFECT OF OIL ON EVAPORATION

In carrying on the observations to determine the effect of oil films on the evaporation from free-water surfaces, two Colorado land pans were installed under identical conditions at the hydraulic laboratory at Fort Collins, Colo. Both were filled with water, and the surface

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<sup>2</sup> Reference is made by number (italic) to Literature Cited p. 729.

<sup>3</sup> This relation is expressed by the formula:

$E = (1.465 - 0.0186B) (0.44 + 0.118W) (e_s - e_d)$ , in which

$E$  = evaporation in inches per 24 hours,

$B$  = mean barometric pressure in inches of mercury at 32° F.,

$W$  = mean ground wind in miles per hour,

$e_s$  = mean vapor pressure of saturated vapor at temperature of water surface, in inches of mercury, and

$e_d$  = mean vapor pressure of saturated air at the temperature of dewpoint, in inches of mercury.

The formula gives the evaporation from U. S. Weather Bureau, class A, land pans and floating pans, Colorado land pans, U. S. Bureau of Plant Industry land pans, U. S. Geological Survey floating pans, and other similar pans when the required data are substituted in it. For Fort Collins, Colo., elevation 5,000 feet, the factor  $(1.465 - 0.0186B)$  is 1.000.

of one was covered with a film of oil. City water, which contained approximately 50 parts per million of solids, was used to fill the tanks. A comparison of the evaporation from the two tanks showed the effect of the oil film. The evaporation tanks were approximately 36 inches square and 18 inches deep, and were sunk in the ground within 1 inch of their tops. Both tanks were painted black with asphaltic paint. The water in the tanks was maintained at approximately the ground level, and a complete record of the meteorological conditions and of the evaporation was taken for each tank. The records consisted of the air and water temperature, the psychrometer reading and the evaporation loss for each tank, and the wind velocity and rainfall records which were the same for both tanks. The

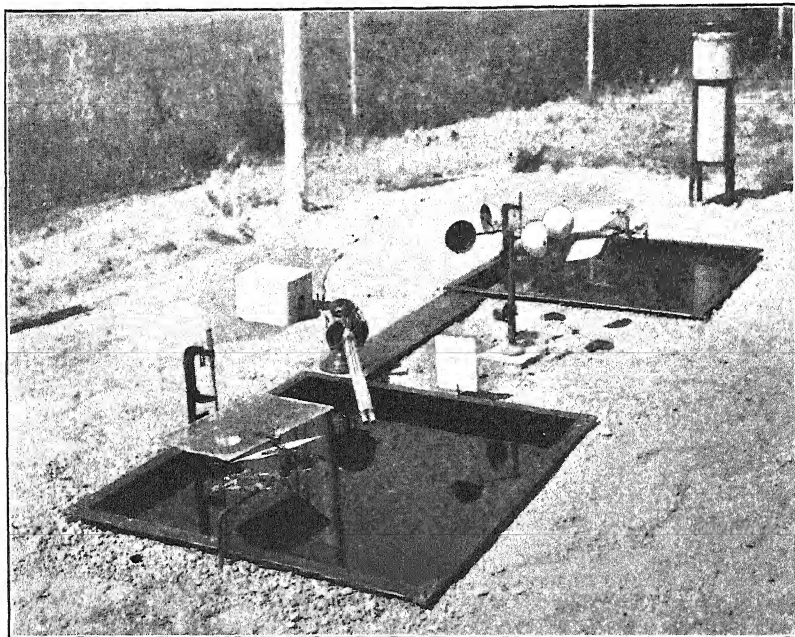


FIGURE 1.—Apparatus used in making tests on the effect of oil films on evaporation

evaporation tanks and the equipment used in making the observations are shown in Figure 1.

The water temperatures were taken at approximately one-half inch beneath the water surface, and the air temperatures were taken at about 1 inch above the water surface in each tank. The thermometers used in making the readings were of good quality and were graduated in degrees and in half degrees. They were compared with a thermometer graduated in tenths of a degree which had been calibrated by the Bureau of Standards, and were found to be quite accurate.

The wet and dry bulb readings for determining the vapor pressure of the water were made with a portable motor-driven aspiration psychrometer, as shown in Figure 1. The instrument was set so the air was drawn from a point about 1 inch above the water surface. This instrument had previously been compared with a calibrated psychrometer and was known to give accurate readings.

The evaporation loss was measured with micrometer hook gages reading to one-thousandth of an inch. These gages had a range of 1 inch, and consequently the variation in the level of the water was limited to this amount. Stilling wells were used in connection with the hook gages.

A standard Weather Bureau anemometer of the 4-cup Robinson type was used for determining the wind velocity. It was placed so that the cups were 18 inches above the ground, and in order to determine the ground velocity a conversion diagram, obtained by comparing the velocity at the 18-inch level with that at the ground level, was used.

The precipitation was measured in a standard Weather Bureau type rain gage which was located near the tanks, as shown in Figure 1.

The observations on the tanks were started on July 18, 1929, and were continued until October 28, 1929. The readings were taken twice daily: Between 7 and 8 a. m. and between 5 and 6 p. m. From these readings, the means of 24-hour periods, which began on the morning of one day and ended on the morning of the next, were computed. A summary of the results is given in Table 1.

Before the tests on the effect of oil on evaporation were started, observations were made under identical conditions on the evaporation from the free-water surfaces of the tanks to determine whether the rate of evaporation was the same from both tanks. These tests covered a period of 10 days, and showed (Table 1) that under identical conditions the rates of evaporation agreed quite closely, the mean difference for the period being only 2 per cent.

TABLE 1.—*Observed and computed evaporation from oil-covered water and from water in Colorado sunken tanks, the ratios between the evaporation from oil-covered water and from water, and the pertinent meteorological data at Fort Collins, Colo., 1929*

[Evaporation computed by formula  $E = (1.465 - 0.0186B)(0.44 + 0.118W)(t_s - e_d)$ ]

Type of surface and tank used	Period	Mean temperature		Mean difference in vapor pressure	Mean ground wind velocity per hour	Precipitation total	Mean evaporation per 24 hours		Ratio of oil-film evaporation to water evaporation observed <sup>a</sup>
		Air	Water				Observed	Computed <sup>a</sup>	
	1929	° F.	° F.	Inch of mercury	Miles	Inch	Inch	Inch	
Water, east tank.....	July 18 to	75.9	76.6	0.390	0.65	0.104	0.203	0.202	-----
Water, west tank.....	July 26.....	76.6	76.1	.376	.65	.104	.199	.194	-----
Water, east tank, 0.0002-inch oil film <sup>b</sup> .....	July 27 to	76.4	76.4	.467	1.08	0	.294	.296	-----
	Aug. 1.....	76.5	77.1	.481	1.08	0	.266	.275	0.919
Water, east tank, 0.002-inch oil film <sup>b</sup> .....	Aug. 13 to	77.7	75.5	.450	.63	.025	.227	.232	-----
	Aug. 21.....	78.7	83.7	.752	.63	.025	.074	.386	.333
Water, east tank, 0.002-inch oil film <sup>b</sup> .....	Aug. 22 to	73.7	73.7	.345	.91	.240	.194	.188	-----
	Aug. 29.....	75.2	79.4	.532	.91	.240	.080	.290	.440
Water, east tank, 0.004-inch oil film <sup>b</sup> .....	Sept. 10 to	57.5	60.2	.214	.90	.258	.116	.118	-----
	Oct. 5.....	59.3	64.8	.322	.90	.258	.026	.177	.279
Water, east tank, 0.002-inch oil film <sup>c</sup> .....	Oct. 6 to	46.8	50.9	.174	1.00	.245	.106	.097	-----
	Oct. 28.....	47.6	52.9	.208	1.00	.245	.065	.116	.597

<sup>a</sup> The computed evaporation and the ratio of the evaporation from the oil-covered water surface to that from the water surface are the means of the daily values.

<sup>b</sup> Transformer oil.

<sup>c</sup> Engine oil, medium.

After these tests had been completed, a film of transformer oil was added to one of the tanks. The first application consisted of 4 cc, which was equivalent to 0.0002 inch on the water surface. When first applied, the oil collected in pools on the water surface, and it took several days for it to spread out evenly. The effect of the film was so slight, however, that the surface was skimmed to remove the oil and then 40 cc of transformer oil was added, which was equivalent to 0.002 inch. This oil was immediately dissipated by the heavy rain occurring at the time, and it was not until the rainfall ceased, when another application of the same amount of oil was made, that it was possible to determine the effect of the oil film. The 40 cc of oil caused a definite reduction in the evaporation, as shown in Table 1, but the effectiveness decreased as a result of the gradual disappearance of the oil film, and by the end of a week the oil was only slightly effective in reducing the evaporation. As soon as the evaporation from the oil surface was nearly equal to that from the free-water surface, the remaining oil was skimmed off and new oil added.

Several applications of oil were made at this rate, but for only two of these were the results free from the effect of heavy rains, which caused the oil to splash out of the tank and, in some cases, to overflow. For the two series of observations which were not affected by rains, the ratios of the evaporation from the oil-covered water surface to that from the free-water surface were 0.333 and 0.440, as shown in Table 1. An application of 80 cc of transformer oil, or a depth of approximately 0.004 inch, was more effective in reducing the evaporation and remained on the water surface longer. In this case, the ratio of the evaporation from the oil-covered surface to that from the water surface was 0.279 and this application of oil lost its effectiveness after 26 days, whereas the smaller quantity of oil was no longer effective after 10 days.

In order to test the effect of films of oil heavier than transformer oil, 40 cc of engine oil, medium grade, was applied to one of the tanks. The engine oil did not reduce the evaporation as much as the transformer oil, but it was not dissipated as rapidly and consequently was effective in reducing the evaporation for a longer period. As shown in Table 1, the ratio of the evaporation from the oil-covered water to that from the free-water surface was 0.597. The difference in the effect of the two different types of oil is probably due partly to the fact that the engine oil was applied late in the season when the evaporation from the free-water surface was small.

From the foregoing tests it is evident that it is possible to reduce the evaporation materially for short periods by the application of oil to the water surface. However, the rapidity with which the oil film disappears in a rainstorm, and the fact that the film is soon dissipated, even in dry weather, seems to make doubtful the economic feasibility of using oil for reducing evaporation from large water surfaces.

The formula

$$E = (1.465 - 0.0186B) (0.44 + 0.118W) (e_s - e_d)$$

shows the relation between the evaporation from a free-water surface and the various meteorological factors. In order to determine whether the same relation held for these experiments, the observed

meteorological data were substituted in the formula. The results are given in Table 1.

A study of the daily mean observed and computed evaporation from the free-water surface for each series of observations shows that the results agree quite closely, as a rule. The mean values for the entire period (not shown) are almost identical, the difference being only 2.9 per cent. The computed evaporation from the oil-covered tank, however, is greatly in excess of the observed evaporation and is considerably greater also than the observed evaporation from the free-water surface. That the computed evaporation from the oil-covered tank should exceed the observed evaporation is to be expected because of the retarding effect of the oil on evaporation, but the reason that it exceeds the evaporation from the free-water surface is that the temperature of the oil-covered water is materially higher than that in the free-water tank. This probably is due to the insulating effect of the oil, and to the reduction in the cooling of the water resulting from the smaller evaporation. The oil may also absorb more heat from the sun. The higher temperature increases the difference in vapor pressure, and since the computed evaporation is proportional to the difference in vapor pressure, it will obviously be greater than the observed evaporation.

#### EFFECT OF SODIUM CHLORIDE ON EVAPORATION

The effect of sodium chloride on evaporation was studied by comparing the evaporation from sodium chloride solutions of different concentrations with the evaporation from water under similar conditions. Three tanks identical in every respect, approximately 36 inches square and 18 inches deep, were used in carrying on the experiments. Each tank was painted black with asphaltic paint and was sunk in the ground to within 1 inch of the top, and the water level was maintained at approximately the ground level. The maximum variation in the water level was 1 inch, and as soon as the water reached the lower level the tanks were refilled. The evaporation tanks and the auxiliary equipment are shown in Figure 2.

The same observations were taken, the same instruments used, and the same procedure followed as in the experiments on the effect of oil on evaporation. One change, however, was made in the procedure when observations were taken on the more concentrated solutions because it was found that the density of the solutions in the stilling wells for the hook gages differed from that in the tanks, and, consequently, the water stood at a different level in the stilling wells than in the tanks. An attempt was made to correct this condition by making the inlets to the stilling wells larger, but this did not prove to be effective in eliminating the difficulty, so the stilling wells were removed entirely. Although the gages could not be read as accurately without the stilling wells, it was thought that the readings would be closer to the true conditions than they would if the stilling wells were used. A summary of the results of the observations is shown in Table 2.

Before the sodium chloride was added to the water, a series of observations covering 10 days was made to see whether the evaporation was the same from all the tanks when filled with water. As Table 2 shows, the evaporation from the different tanks for the 10-day period was nearly the same. The daily mean rates were not so

nearly similar, but the differences tended to balance each other, as is shown by the means for the period.

After completing the tests to determine whether the evaporation from all the tanks was the same when filled with water, common salt (NaCl) was added to the water in two of the tanks. The amounts of sodium chloride added were, respectively, 2 per cent and 5 per cent of the weight of the water in the tanks. Because the total weight of the solution was slightly greater than the weight of the water alone, the true percentage of salt in a given weight of solution was slightly less than that indicated.

The first series of tests on the comparison between the evaporation from water and from 2 and 5 per cent sodium chloride solutions was started June 19, 1930, and continued until July 22, 1930. At the end of this period, the 2 per cent sodium chloride solution was trans-

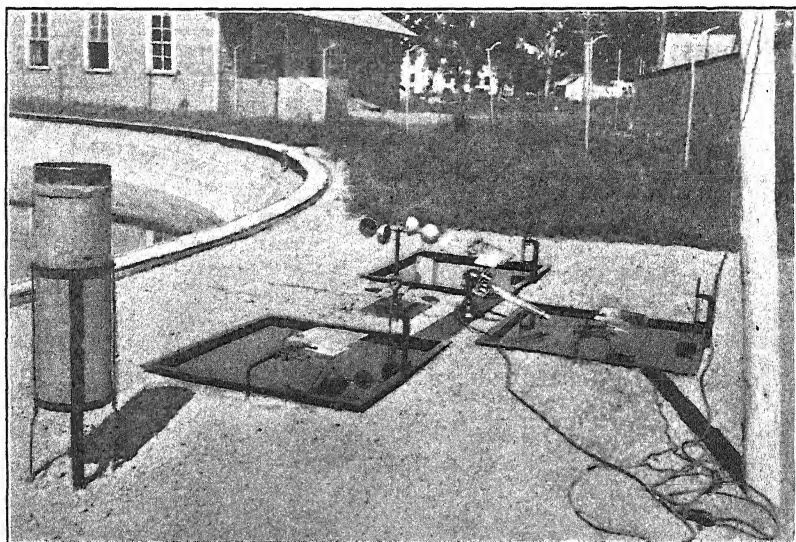


FIGURE 2.—Evaporation tanks and auxiliary equipment for testing the effect of sodium chloride on the evaporation from water

ferred to the tank which had been filled with water, and the tank which had held the 2 per cent solution previously was filled with fresh water. The experiments under this condition were continued until September 2, 1930, except for the period from August 11 to 18, 1930, during which the observations were discontinued on account of the heavy rainfall.

The results of the comparison for the first period show (Table 2) that the mean daily evaporation from the solutions increased as the salt concentration increased, or just the opposite of what was anticipated. The results of the comparison for the second period, when the fresh water and the 2 per cent sodium chloride solution were interchanged show that the evaporation from the salt solutions was less than that from the pure water; nevertheless the evaporation from the 5 per cent solution was greater than the evaporation from the 2 per cent solution.

For the entire period of 67 days, the mean daily evaporation from the different solutions, disregarding the position of the tanks, was,

respectively, 0.224, 0.219, and 0.223 inch for the pure water, the 2 per cent and the 5 per cent solutions. These results show that the evaporation was slightly less from the sodium chloride solutions, but still the loss from the 5 per cent solution was greater than that from the 2 per cent solutions, which indicates that the small differences noted are not significant.

While these observations were being carried on, it was observed that the temperatures of the solutions in the tanks containing sodium chloride seemed to be higher than the temperature of the water in the water tank. In the first series of tests the temperatures of the salt solutions were definitely higher than those of the water (Table 2), but in the second series the differences were insignificant. It is doubtful therefore whether the erratic results may be attributed entirely to the small differences in temperature.

TABLE 2.—*Observed and computed evaporation from water and from sodium chloride solution in Colorado sunken tanks, the ratios between the evaporation from sodium chloride solutions and from water, and the pertinent meteorological data at Fort Collins, Colo., 1930*

[Evaporation computed by formula  $E = (1.465 - 0.0186B) (0.44 + 0.118W) (e - e_a)$ ]

Type of solution and tank used	Period	Mean temperature		Mean difference in vapor pressure	Mean ground wind velocity per hour	Precipitation total	Mean evaporation per 24 hours		Ratio of solution evaporation to water evaporation observed <sup>a</sup>
		Air	Water				Observed	Computed <sup>a</sup>	
		° F.	° F.	Inch of mercury	Miles	Inches	Inch	Inch	
Water, west tank.....	June 8 to June 17.	70.8	70.6	0.425	1.18	0.645	0.259	0.248	
Water, south tank.....		70.4	70.6	.421	1.18	.645	.256	.246	
Water, east tank.....	June 19 to July 22.	69.8	71.0	.430	1.18	.645	.262	.252	
Water, south tank.....		75.8	74.3	.439	1.11	.956	.250	.251	
2 per cent NaCl, west tank.....	July 24 to Sept. 2.	76.0	74.4	.445	1.11	.956	.254	.255	1.022
5 per cent NaCl, east tank.....		75.0	75.3	.442	1.11	.956	.255	.254	1.009
Water, west tank.....	June 19 to Sept. 2.	74.4	73.6	.373	.84	1.966	.197	.202	
2 per cent NaCl, south tank.....		74.1	73.3	.350	.84	1.966	.182	.189	.925
5 per cent NaCl, east tank.....	Sept. 4 to Nov. 15.	72.8	73.7	.350	.84	1.966	.190	.190	.955
Water.....		75.1	73.9	.406	.98	2.922	.224	.227	
2 per cent NaCl.....	Sept. 2.	75.1	73.8	.398	.98	2.922	.219	.223	.974
5 per cent NaCl.....		74.0	74.5	.397	.98	2.922	.223	.222	.983
Water, west tank.....	Sept. 4 to Nov. 15.	50.2	52.4	.190	1.11	.574	.115	.108	
10 per cent NaCl, east tank.....		50.0	52.3	.170	1.11	.574	.109	.096	.930
20 per cent NaCl, south tank.....		49.9	52.2	.138	1.11	.574	.092	.079	.776

<sup>a</sup> The computed evaporation and the ratio of the evaporation from the sodium chloride solutions to that from the water are the means of the daily values.

Because the differences between the evaporation from water and that from 2 and 5 per cent solutions of sodium chloride were not significant, the salt content in the tanks was increased to 10 and 20 per cent in order to magnify the effects of the solutions on evaporation sufficiently so that they would not be overshadowed by observational errors. This was done by adding enough sodium chloride to that already in the tanks to obtain the desired concentrations. The tests on the concentrated solutions were started September 4, 1930, and were continued until November 15, 1930, when it became necessary to discontinue them on account of the cold weather.



Some difficulty was experienced with the 20 per cent solution because of its tendency to increase in concentration near the bottom. This phenomenon was detected when it was noticed that foreign material heavier than water sank to a definite level in the tank. Occasional stirring, however, corrected this condition.

The results obtained with the 10 per cent and 20 per cent solutions (Table 2) show that the sodium chloride materially reduced the rate of evaporation, and the smallest evaporation was from the tank containing the most concentrated solution.

The ratios of the daily evaporation from each of the tanks containing sodium chloride solutions to that from the tank containing water were computed, and the mean ratios for each series of observations are given in Table 2. These ratios were approximately 0.97 for the 2 per cent solution, 0.98 for the 5 per cent solution, 0.93 for the 10 per cent solution, and 0.78 for the 20 per cent solution. Experiments by Harris and Robinson (2) on the effect of common salt on the evaporation from glass tumblers, under laboratory conditions without wind, showed rates of evaporation from 10 and 20 per cent solutions, respectively, to be 79 per cent and 68 per cent of that from distilled water. Experiments by Briggs (1, p. 193-195) and by Lee (3, p. 332-333) showed similar results.

The agreement between the results obtained by the different experimenters is not close, but all the observations show that evaporation from the more concentrated salt solutions is definitely less than from weak solutions or from pure water.

In order to determine whether the relation between the evaporation from the different solutions of sodium chloride to the meteorological factors was the same as it was for water, the meteorological data were substituted in the formula

$$E = (1.465 - 0.0186B) (0.44 + 0.118W) (e_s - e_a),$$

which shows the relation between the meteorological factors and the evaporation from water in tanks. Before substituting the observed data in the formula, it was necessary to find the reduction in vapor pressure of the solution due to the salt in the water. According to Raoult's law (5, p. 57-59) the ratio of the vapor pressure of a solution to that of water is independent of the temperature and pressure. This fact is confirmed by the vapor-pressure data of the International Critical Tables (4, p. 370-371). In so far as determining the numerical values of the ratios is concerned, this law holds only for very dilute solutions and can not be used directly in the computation of the vapor pressures of concentrated solutions, but since the vapor pressures at the boiling point of water have been experimentally determined (7, p. 182) for different concentrations of various salts, the ratios can be determined by dividing the vapor pressure of the salt solution at the boiling point by the vapor pressure of the water at the same temperature. The vapor pressure of the solution at any other temperature is this ratio times the vapor pressure of water at the same temperature.

The values of the ratios computed on this basis are as follows: 2 per cent solution 0.989, 5 per cent solution 0.972, 10 per cent solution 0.948, and 20 percent solution 0.880. In computing the ratios, the true percentage of salt in the 10 and 20 per cent solutions, as determined by



the weight of salt in a given weight of solution, was used, but in the 2 and 5 per cent solutions the nominal percentage was used because the density of these solutions was not materially different from that of water. It will be noted that the reduction in vapor pressure was small for the 2 and 5 per cent solutions, which is probably the reason why the small percentages of salt had but a negligible effect on the evaporation.

The evaporation computed by substituting these data in the evaporation formula agrees quite well with the observed evaporation except in the last series of tests in which the 10 and 20 per cent solutions were used. Table 2 shows that the observed evaporation was greater than the computed evaporation for both the 10 and the 20 per cent solutions and also for the water, but the difference was greatest for the 20 per cent solution. The percentages of deviation are shown in Table 3.

TABLE 3.—*Deviation of computed from observed evaporation in the observations with solutions of sodium chloride*

Type of solution and tank	Mean evaporation per 24 hours		Deviation
	Observed	Computed <sup>a</sup>	
Water series:	<i>Inch</i>	<i>Inch</i>	<i>Per cent</i>
West tank.....	0.259	0.248	-4.2
South tank.....	.256	.246	-3.9
East tank.....	.262	.252	-3.8
2 and 5 per cent NaCl series:			
Water.....	.224	.227	+1.3
2 per cent.....	.219	.223	+1.8
5 per cent.....	.223	.222	-0.4
10 and 20 per cent NaCl series:			
Water.....	.115	.108	-6.1
10 per cent.....	.109	.096	-11.9
20 per cent.....	.092	.079	-14.1

<sup>a</sup> Computed by formula  $E = (1.465 - 0.0186E) (0.44 + 0.118W) (e_s - e_a)$ .

Although the percentages of deviation in the computed results for the 10 and 20 per cent solutions are rather large, it should be noted that the percentage for the water also is considerable and of the same sign. When this fact is taken into consideration, the differences between the observed and the computed rates of evaporation from the concentrated salt solutions do not appear remarkable; consequently it seems that the effects of meteorological factors upon evaporation from salt solutions are the same as upon evaporation from water.

#### EFFECT OF SODIUM SULPHATE ON EVAPORATION

The observations on the evaporation from solutions of sodium sulphate, commonly known as white alkali, were made with the same equipment as was used in making the observations on the effect of sodium chloride on evaporation. The same observations were taken and the same procedure was followed, except that the water-temperature readings were taken by holding the thermometer in each tank at the proper depth and the air temperatures were taken from the dry-bulb readings of the psychrometer. In conducting these tests, however, four tanks were used instead of three, and none of the tanks was painted at the beginning of the series of tests. No stilling wells were used in connection with the hook gages on the tanks because

it was assumed that the stilling wells would introduce errors into the readings on account of the difference in density of the solution in the tanks and in the stilling wells, as was noted in the case of the tests on sodium chloride solutions. Commercial anhydrous sodium sulphate was used in making the solutions. This salt is only moderately soluble in water at ordinary temperatures, and for this reason 10 per cent was the maximum concentration of solution investigated, but observations were also made on 2 and 5 per cent solutions to find out how effective the less concentrated solutions were in reducing the evaporation. As in the previous tests, the comparisons were made with the evaporation from water.

The use of four tanks instead of three made it necessary to rearrange the tanks. The new arrangement is shown in Figure 3. On account

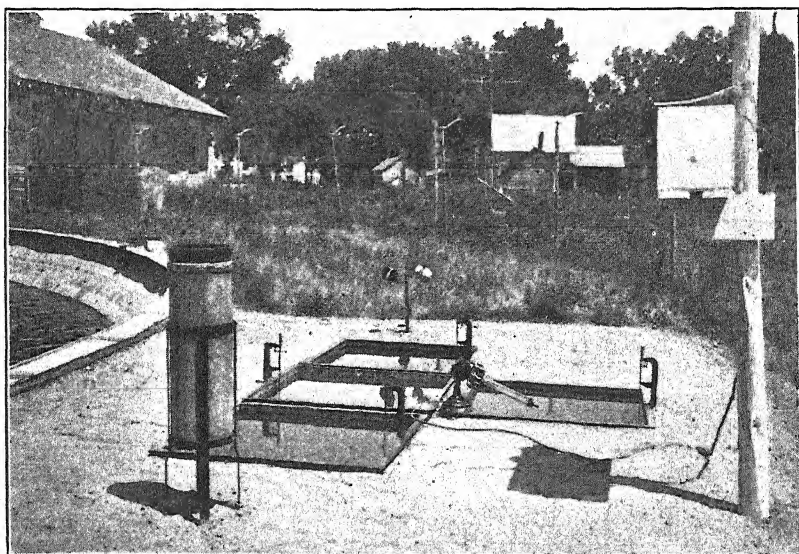


FIGURE 3.—Evaporation tanks arranged for making tests on the effect of sodium sulphate on evaporation from water

of the change in the position of the tanks, a preliminary series of observations was made when all the tanks were filled with water to determine whether the evaporation was the same from all of them. This series of observations was started on July 1, 1931, and was continued until August 1, 1931.

During the course of the observations it was noted that evaporation from the center tank of the three in a row was consistently less than that from the other tanks, and that the temperature of the water was also lower. This was attributed to the fact that the tanks had been left unpainted, because in the tests with sodium chloride solutions it was found that the action of the salt solution caused the paint to peel off, and as a result the interiors of the tanks were not of a uniform color. On July 16, 1931, the inside of each tank was painted with black asphaltic paint, and from then on the interiors of the tanks were uniform in color.

A summary of the results of these observations is given in Table 4. As shown in this table painting the tanks did not make the results

check any more closely. For the entire period that the tanks were filled with water only, the evaporation was, respectively, 0.296, 0.288, 0.304, and 0.294 inch. Comparison of these values with the mean for all the tanks shows that the evaporation from the center tank was 2.7 per cent low and that from the east tank was 2.7 per cent high, but the evaporation from the west and south tanks was almost identical with the mean value for all the tanks.

TABLE 4.—*Observed and computed evaporation from water and from sodium sulphate solutions in Colorado sunken tanks, the ratios between the evaporation from sodium sulphate solutions, and from water, and the pertinent meteorological data at Fort Collins, Colo., 1931*

[Evaporation computed by formula  $E=(1.465-0.0186B)(0.44+0.0118W)(e_s-e_d)$ ]

Type of solution and tank	Period	Mean temperature		Mean difference in vapor pressure	Mean ground wind velocity per hour	Precipitation total	Mean evaporation per 24 hours		Ratio of solution evaporation to water evaporation observed <sup>a</sup>
		Air	Water				Observed	Computed <sup>a</sup>	
		° F.	° F.	Inch of mercury	Miles	Inches	Inch	Inch	
Water, west tank.....	July 2 to 14.....	72.4	72.8	0.466	1.91	Trace	0.318	0.287	-----
Water, center tank.....		72.2	72.4	.462	1.91	Trace	.308	.284	-----
Water, east tank.....		72.4	73.1	.480	1.91	Trace	.326	.294	-----
Water, south tank.....		72.1	72.8	.474	1.91	Trace	.313	.291	-----
Water, west tank.....	July 16 to 31 <sup>b</sup> .....	78.1	76.6	.469	1.60	0.120	.279	.284	-----
Water, center tank.....		77.7	76.2	.463	1.60	.120	.272	.279	-----
Water, east tank.....		78.0	76.6	.476	1.60	.120	.287	.286	-----
Water, south tank.....		77.8	76.5	.475	1.60	.120	.278	.286	-----
Water, west tank.....	July 2 to 31.....	75.6	74.9	.468	1.74	.120	.296	.285	-----
Water, center tank.....		75.2	74.6	.462	1.74	.120	.288	.281	-----
Water, east tank.....		75.4	75.0	.478	1.74	.120	.304	.290	-----
Water, south tank.....		75.2	74.8	.474	1.74	.120	.294	.288	-----
Water, west tank.....	Aug. 1 to 31.....	71.1	72.2	.412	1.12	.886	.220	.231	-----
2 per cent Na <sub>2</sub> SO <sub>4</sub> , west tank.....		71.2	72.0	.385	1.12	.886	.227	.216	1.030
5 per cent Na <sub>2</sub> SO <sub>4</sub> , center tank.....		71.1	71.7	.380	1.12	.886	.209	.213	.955
10 per cent Na <sub>2</sub> SO <sub>4</sub> , east tank.....		71.2	72.1	.391	1.12	.886	.215	.219	.984
Water, south tank.....	Sept. 1 to 30.....	64.4	65.4	.320	1.29	.369	.186	.188	-----
2 per cent Na <sub>2</sub> SO <sub>4</sub> , west tank.....		64.4	65.2	.313	1.29	.369	.191	.182	1.020
5 per cent Na <sub>2</sub> SO <sub>4</sub> , center tank.....		64.3	65.1	.298	1.29	.369	.179	.174	.945
10 per cent Na <sub>2</sub> SO <sub>4</sub> , east tank.....		64.4	65.2	.306	1.29	.369	.185	.178	.972
Water, south tank.....	Oct. 1 to 31.....	49.1	50.7	.162	1.78	1.013	.100	.098	-----
2 per cent Na <sub>2</sub> SO <sub>4</sub> , west tank.....		49.3	50.5	.159	1.78	1.013	.108	.096	1.087
5 per cent Na <sub>2</sub> SO <sub>4</sub> , center tank.....		49.2	50.4	.152	1.78	1.013	.108	.092	1.028
10 per cent Na <sub>2</sub> SO <sub>4</sub> , east tank.....		49.1	50.4	.151	1.78	1.013	.106	.092	.974
Water, south tank.....	Aug. 1 to Oct. 31.....	61.5	62.7	.298	1.40	2.268	.168	.172	-----
2 per cent Na <sub>2</sub> SO <sub>4</sub> , west tank.....		61.6	62.6	.285	1.40	2.268	.175	.164	1.045
5 per cent Na <sub>2</sub> SO <sub>4</sub> , center tank.....		61.5	62.4	.277	1.40	2.268	.165	.160	.976
10 per cent Na <sub>2</sub> SO <sub>4</sub> , east tank.....		61.5	62.5	.282	1.40	2.268	.168	.163	.977

<sup>a</sup> The computed evaporation and the ratio of the evaporation from the sodium sulphate solutions to that from the water are the means of the daily values.

<sup>b</sup> Tanks cleaned and painted with asphaltic paint on July 16.

The sodium sulphate was added to the tanks on August 1, 1931. The amount of salt used was determined by taking the indicated percentage (2, 5, or 10) of the weight of water in each tank as determined from its volume. This method gave only approximate results. The true percentages of sodium sulphate in the solutions were determined from their temperatures and densities. No sodium sulphate was added to the south tank because the comparative results on the evaporation from water were to be obtained from it.

The observations on the sodium sulphate solutions were started on August 1, 1931, and were continued until November 1, 1931. The results are given in Table 4, which is a summary by months of the

daily observations. The results in Table 4 show that the evaporation from the 10 per cent solution was greater than it was from the 5 per cent solution in August and September, and less in October, but in every case the differences were small. In this connection it should be recalled, however, that when these tanks were filled with water, the evaporation from all the tanks was not the same, the center tank being consistently low and the east tank consistently high. As the 5 per cent solution was in the center tank and the 10 per cent solution was in the east tank, one reason for the unexpected results in the August and September records becomes apparent.

No doubt the principal reason that the same results were not obtained in October was that conditions during this period were unusual. The temperatures in the tanks dropped so low that the 10 per cent solution reached the saturation point and started to crystallize. Some of the crystals formed as flakes on the surface of the solutions and probably reduced the evaporation to some extent by reducing the evaporating area. When the temperature dropped considerably below freezing, the crystallization of the solution apparently was carried below the saturation point, for it was observed that ice formed only on the tank containing the 10 per cent solution and on the tank containing water. Although all the tanks containing the sodium sulphate solutions were stirred each morning and evening when the readings were taken, the fact that ice formed only on the tank containing the 10 per cent solution and on the tank containing water indicated that under these conditions, at least, the surface of the solution was nearly free from sodium sulphate.

At the end of the season, observations made on the densities of the solutions showed that on account of the crystallization of the sodium sulphate, the specific gravity of the solution in the tank containing 10 per cent of sodium sulphate had dropped until it was only slightly higher than the specific gravity of the 5 per cent solution. Under normal conditions this should have increased the evaporation, but the accelerating effect was probably counterbalanced by the retarding effect of the ice in the tank. As the weather grew colder, there was also a tendency for the solutions to crystallize around the edges of the tanks. The capillary action kept these crystals wet, which increased the area of the evaporating surface and consequently increased the evaporation. This is probably the reason why the evaporation from the solutions was consistently greater than it was from the water in October. In this connection it should, however, be mentioned that the ice in the water tank during the latter part of October caused a decrease in the evaporation, which would also make the difference between the evaporation from the water and from the solutions greater.

For the entire period of three months, the mean daily evaporation from the water and from the 2, 5, and 10 per cent solutions was, respectively, 0.168, 0.175, 0.165, and 0.168 inch, and the ratios of the evaporation from the different solutions to that from water were, respectively, 1.045, 0.976, and 0.977. These results are inconsistent because the evaporation from the tank containing water is exactly the same as the evaporation from the tank containing 10 per cent of sodium sulphate and less than the evaporation from the tank containing 2 per cent of sodium sulphate. The differences are small, however, and indicate that the effect of sodium sulphate on the evaporation, for these conditions at least, is negligible (*1, p. 195*). Correct-

ing the results in accordance with the differences found when making the observations on the tanks when all were filled with water would make the results more consistent but would not show that the sodium sulphate affected the evaporation materially.

The relation of the meteorological factors to the evaporation from the sodium sulphate solutions was checked by substituting the observed meteorological data in the formula

$$E = (1.465 - 0.0186B) (0.44 + 0.118W) (e_s - e_d),$$

which shows the relation between the evaporation from free-water surfaces of this type and the meteorological data. If the same relation holds, then the evaporation computed by the formula should equal the observed evaporation. Before substituting the data in the formula, it was necessary to compute the vapor pressure of the solutions. This was done in the same manner as for the sodium chloride solutions and was based on the true percentages of sodium sulphate in the solutions, as determined from their densities. The results obtained by substituting these data in the formula are given in Table 4 and are summarized in Table 5.

TABLE 5.—*Deviation of computed from observed evaporation in the observations with solutions of sodium sulphate*

Type of solution, month and tank	Mean evaporation per 24 hours		Deviation
	Observed	Computed <sup>a</sup>	
Water series:	<i>Inch</i>	<i>Inch</i>	<i>Per cent</i>
July:			
South tank.....	0.294	0.288	-2.0
West tank.....	.296	.285	-3.7
Center tank.....	.288	.281	-2.4
East tank.....	.304	.290	-4.6
2, 5, and 10 per cent solutions:			
August:			
Water.....	.220	.231	+5.0
2 per cent.....	.227	.216	-4.8
5 per cent.....	.209	.213	+1.9
10 per cent.....	.215	.219	+1.9
September:			
Water.....	.186	.188	+1.1
2 per cent.....	.191	.182	-4.7
5 per cent.....	.179	.174	-2.8
10 per cent.....	.185	.178	-3.8
October:			
Water.....	.100	.098	-2.0
2 per cent.....	.108	.096	-11.1
5 per cent.....	.108	.092	-14.8
10 per cent.....	.106	.092	-13.2
August, September, and October means:			
Water.....	.168	.172	+2.4
2 per cent.....	.175	.164	-6.3
5 per cent.....	.165	.160	-3.0
10 per cent.....	.168	.163	-3.0

<sup>a</sup> Computed by formula  $E = (1.465 - 0.0186B) (0.44 + 0.118W) (e_s - e_d)$ .

These results show that it was possible to compute the evaporation from sodium sulphate solutions by substituting the wind velocity, barometric pressure, and difference in vapor pressure in the formula for the evaporation from water. The agreement between the observed and computed evaporation for the sodium sulphate solutions is not so close as it is for the water, but the errors are not large except for the October observations. For this series, the deviation may be due

to the fact that the solutions started to crystallize on the edges of the tanks, thus increasing the area of the evaporating surface as a result of the capillary action of the crystals. The means for the entire period during which the observations were made on the solutions show a close agreement, but the results are necessarily affected by the large errors occurring in the October observations. If the October observations are disregarded, the results show that the same law holds for both the sodium sulphate solutions and the water.

#### SUMMARY

Observations on the effect of oil films on evaporation show that evaporation is reduced by the presence of the oil film, and that the effectiveness of the film increases with its thickness. Heavy oil apparently did not reduce the evaporation as much as light oil, but this may have been due to the fact that the evaporation from the water surface was small at the time the observations on heavy oil were made. The short time in which the oil films were effective in reducing evaporation, due to dissipation by rainfall, wind, and other causes, indicates that this method is not economically feasible for reducing evaporation from large water surfaces. The relation which holds between the meteorological factors, that is, barometric pressure, wind velocity, and difference in vapor pressure, and the evaporation from free-water surfaces does not hold for water surfaces covered by oil films. The temperatures of oil-covered water surfaces are definitely higher than those of free-water surfaces similarly exposed.

Observations on the evaporation from solutions of sodium chloride of different strengths, when compared with the evaporation from water surfaces, all in exactly similar tanks similarly exposed, show that the evaporation from 2 and 5 per cent solutions does not differ materially from that from water, but that the evaporation from 10 and 20 per cent solutions is definitely less than that from water. For the 10 and 20 per cent solutions the evaporation decreases as the salt concentration increases. For the entire period of the observations, the ratios of the evaporation from the 2, 5, 10, and 20 per cent sodium chloride solutions to that from water were respectively 0.97, 0.98, 0.93, and 0.78.

The relation between the rate of evaporation from water and the meteorological factors evidently holds also in general for sodium chloride solutions. If the vapor pressure is corrected for the effect of the sodium chloride, the computed rates of evaporation agree well with the observed rates, but the agreement is closer for the weaker than for the more concentrated solutions.

The evaporation from the sodium sulphate solution agrees quite closely with the evaporation from water when exposed under similar conditions. For the entire period during which the observations were taken, the ratio of the evaporation from the 2 per cent solution to that from water was 1.04, and from the 5 per cent and 10 per cent solutions was 0.98. These ratios show no definite trend, but observations made on the same tanks when filled with water showed that the evaporation from the tank which was later filled with the 5 per cent sodium sulphate solution was low, and that the evaporation from the tank which was later filled with the 10 per cent solution was high.

In general, the evaporation from the sodium sulphate solutions follows the same law as the evaporation from water. In computations of evaporation, of course, the vapor pressure of the water in the solution should be corrected for the effect of the salt. As in the case of the sodium chloride solutions, the agreement between observed and computed evaporation was closer for the larger than for the smaller rates.

The evaporation law expressed by the formula

$$E = (1.465 - 0.0186B) (0.44 + 0.118W) (e_s - e_a)$$

was found to be true for the evaporation from the tanks containing water throughout the entire series of tests.

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# THE EFFECT OF LATITUDE, LENGTH OF GROWING SEASON, AND PLACE OF ORIGIN OF SEED ON THE YIELD OF COTTON VARIETIES<sup>1</sup>

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## INTRODUCTION

The determination of the inherent yielding ability of seed from different cotton varieties under local field conditions is one of the projects in agronomy underway at all of the Cotton Belt experiment stations. According to Cook (2, p. 11)<sup>2</sup> there are probably from 200 to 300 named varieties available for testing. These so-called varieties were developed in various ways, and under a wide range of growing conditions. The multiplicity of environmental factors affecting yield, such as soil, prevalence of insects and diseases, amount of light, sunshine, heat, moisture, length of growing season, place of testing, and the conditions under which the seed were produced, make an analysis of cotton varietal yields difficult even for a single location.

Very little work has been reported on the effect of latitude, length of growing season, and place of origin of seed on the yield of cotton varieties. Winters, as reported by Brown (1, p. 236), selected and increased a strain of the King variety of cotton at West Raleigh, N. C., for two years. In 1916 the selected seed was divided, and one half was grown at West Raleigh and the other half at Agricultural College, Miss. During 1917 and 1918, the Mississippi-grown seed was compared with the North Carolina-grown seed at both places. At Agricultural College, Miss., it was found that seed grown there produced taller plants and more bolls than did seed from the original lot grown at West Raleigh, N. C. In the corresponding tests made in North Carolina, Mississippi-grown seed also made the best growth and slightly better yields.

## MATERIALS AND METHODS

The yield data for as many varieties and locations as were available for recent years were used in this study. Seasonal averages of not less than three years for lint or seed cotton yields were obtained from published reports or were compiled from them and from type sheets furnished by those in charge of cotton-variety testing in Texas, Oklahoma, Missouri, Arkansas, Louisiana, Mississippi, Tennessee, Alabama, Georgia, and South Carolina. The source given for each variety during the years making up the average yields may not be exact, as in some cases it was necessary to assume that seed was obtained from the same place every year.

The approximate latitude of the places of origin of the seed and of the places where the tests were made was determined from maps (3) giving the latitude to one-half degree, except for Texas where the

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 737.

latitude was given in a whole degree, or approximately 70 miles. The approximate average number of frost-free days, or the average number of days free of killing frost, at the places of origin and places where tested was determined from a map (7, p. 11) prepared by the United States Weather Bureau. The data are not presented for the 37 test locations as the correlations given summarize the results of this phase of the work.

The map (fig. 1) of the United States Cotton Belt shows the location of varietal experiments and the approximate number of annual frost-free days for each location included in the correlation study.

Two simple correlation coefficients were calculated to express mathematically the tendency of varieties with high yields to have places of origin of seed similar in latitude and length of growing season to those where the tests are made. Relationships were studied between the relative yield (highest-yielding variety equaling 100 per cent) of each variety and the difference in latitude, and the difference in the annual number of frost-free days, at each of the 37 test locations, and the place of origin of the seed, of each variety tested. The number of varieties at each location varied from 4 to 26, making it possible to include 406 sets of observations in each correlation study.

In order to get additional information on the adaptation of cotton seed, another study was made in which the yield of a local or unnamed variety in 68 tests was compared with the average yields of introduced seed of named varieties. The results of this study are given in Table 1.

TABLE 1.—Comparison of yield from introduced and locally grown cottonseed in 68 tests at 37 locations

Place tested	Year	Varieties tested	Rank of local seed in yield	Acre yield of—				Difference in favor of local seed	
				Local seed		Average all varieties			
				Lint	Seed cotton	Lint	Seed cotton	Lint	Seed cotton
		<i>Number</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Burdette, Ark. ....	1920	22	1		1,083		866		+217
Fort Smith, Ark. ....	1920	8	1		1,210		1,070		+140
Do. ....	1922	8	4		475		468		+7
Baber, Ark. ....	1920	8	5		887		888		—1
Heber Springs, Ark. ....	1920	8	4		1,427		1,403		+24
Do. ....	1921	10	4		303		335		—32
Blytheville, Ark. ....	1922	8	5		1,537		1,571		—34
Wynne, Ark. ....	1922	8	5		633		706		—73
Waldron, Ark. ....	1922	8	1		528		277		+251
Gregory, Ark. ....	1922	8	1		1,110		998		+112
Do. ....	1923	13	8		273		368		—95
Do. ....	1924	17	15		1,103		1,361		—258
Morrilton, Ark. ....	1920	7	4		1,993		1,917		+76
Brickeys, Ark. ....	1920	8	5		860		893		—33
Dermott, Ark. ....	1920	8	7		790		953		—163
Marianna, Ark. ....	1921	9	3		1,553		1,182		+371
Bryant, Ark. ....	1921	8	5		536		548		—12
Do. ....	1921	7	2		672		516		+156
Magnolia, Ark. ....	1921	8	9		285		359		—74
Bonnerdale, Ark. ....	1922	8	6		334		358		—24
Pocahontas, Ark. ....	1922	8	1		1,300		911		+389
Robroy, Ark. ....	1923	19	13		779		872		—93
Laurel Hill, Fla. ....	1928	5	4		867		989		—122
Do. ....	1930	19	6		314		311		+3
Sylacauga, Ala. ....	1928	26	11		159		152		+7
Do. ....	1929	32	14		269		257		+12
Cedar Bluff, Ala. ....	1928	26	22		325		405		—80
Do. ....	1929	32	5		425		358		+67
Belle Mina, Ala. ....	1928	26	10		456		432		+24
Do. ....	1930	33	16		243		244		—1
Do. ....	1931	28	6		457		408		+49

TABLE 1.—Comparison of yield from introduced and locally grown cottonseed in 68 tests at 37 locations—Continued

Place tested	Year	Varieties tested	Rank of local seed in yield	Acre yield of—				Difference in favor of local seed	
				Local seed		Average all varieties			
				Lint	Seed cotton	Lint	Seed cotton	Lint	Seed cotton
		<i>Number</i>		<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Alexandria, Ala.	1928	26	5	173		145		+28	
Do	1930	33	20	218		226		—8	
Do	1931	28	12	328		323		+5	
Andalusia, Ala.	1928	19	16	283		320		—37	
Do	1929	19	2	343		309		+34	
Do	1930	22	8	174		162		+12	
Do	1931	21	5	519		569		—50	
Crossville, Ala.	1929	32	7	446		417		+29	
Do	1930	33	14	446		428		+18	
Do	1931	28	18	606		628		—22	
Auburn, Ala.	1929	20	15	275		292		—17	
Do	1930	27	8	381		364		+17	
Do	1930	33	12	503		469		+34	
Do	1931	34	3	527		452		+75	
Do	1931	19	6	376		360		+16	
Prattville, Ala.	1929	19	7	382		366		+16	
Do	1930	22	3	212		186		+26	
Do	1930	33	4	212		161		+51	
Do	1931	28	16	504		507		—3	
Do	1931	19	8	496		491		+5	
Benton, Ala.	1929	19	1	131		98		+33	
Do	1930	22	8	394		367		+27	
Do	1931	20	3	426		507		—81	
Headland, Ala.	1929	19	6	385		369		+16	
Do	1930	24	3	628		574		+54	
Do	1931	21	2	274		335		—61	
La Fayette, Ala.	1930	33	13	379		348		+31	
Do	1931	27	23	462		394		+68	
Benton, Ala.	1930	22	10	326		322		+4	
Aliceville, Ala.	1931	19	14	552		529		+23	
Do	1931	28	20	536		569		—33	
Monroeville, Ala.	1931	19	15	366		321		+45	
Gastonburg, Ala.	1931	28	5	448		503		—55	
Rome, Ga.	1931	25	5	390		338		+52	
Fort Valley, Ga.	1931	23	9	573		555		+18	
Carnegie, Ga.	1931	23	11	379		384		—5	
Waynesboro, Ga.	1931	23	17	344		376		—32	
Average		20	8	379±13	893±65	370±13	861±60	9±18	32±88

## EXPERIMENTAL RESULTS

## CORRELATIONS BETWEEN YIELD AND LATITUDE AND YIELD AND NUMBER OF FROST-FREE DAYS

Correlation coefficients of  $-0.143$  and  $-0.118$  were found between relative yield and differences in latitude and differences in number of frost-free days, respectively. While the coefficients are small, the large number of observations justify the conclusion that there is a significant correlation between the yield of cotton varieties and their place of origin.

According to the tables prepared by Wallace and Snedecor (8, p. 63), when the number of observations is 400, the least significant value of  $r$  is 0.098, while 0.128 is the least highly significant value. The negative coefficients show that high relative yield is associated with small differences between the latitude and the annual number of frost-free days for the test locations and the places of origin of seed of the varieties tested.

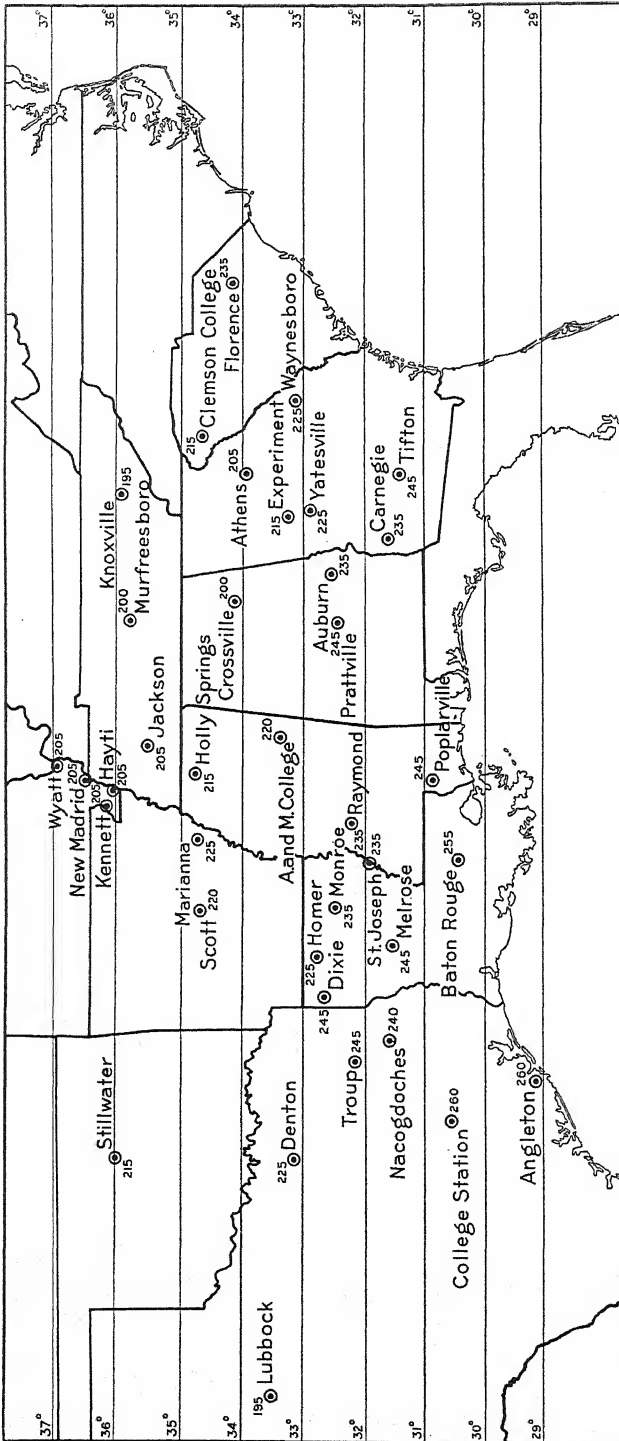


FIGURE 1.—United States Cotton Belt showing location, and approximate number of frost-free days for experiments used in the correlation study

## COMPARISON OF LOCAL AND INTRODUCED COTTONSEED

Table 1 gives the average yield of all named or introduced varieties and the yield and rank of the locally grown cottonseed of unnamed kinds in 68 varietal tests at 37 places in Arkansas, Florida, Alabama, and Georgia. The differences in favor of the yield of local seed for each location is given as well as the average yields for all tests where local and introduced seed are compared. The average acre yields of seed cotton in 23 experiments are reported, and average yields of lint in 45 experiments. The yield of local or home-grown seed exceeded that of all varieties by  $32 \pm 88$  pounds per acre of seed cotton and by  $9 \pm 18$  pounds per acre of lint. In 41, or 60.3 per cent, of the 68 tests tabulated, the locally grown seed outyielded that brought in from other places and latitudes for the experiments. The average number of varieties in each test was 20, and the average rank of local seed in yield was 8.

## DISCUSSION

The correlation coefficient of  $-0.143$  between the relative yield and the difference in latitude of the place of origin and location of the varietal test may be considered highly significant. It shows that there is a strong tendency for cotton to produce high yields when the seed is grown in the same latitude as the place where it is tested. No data are available as to the length of time necessary for this climatic adaptation, but it is probable that at least three or four years are necessary.

The correlation coefficient of  $-0.118$  between the relative yield and the difference in frost-free days at the place of origin and the location of the varietal test may be considered as significant. This coefficient indicates that the number of frost-free days where varieties originate has about as much effect on their yielding ability as the latitude at the place of origin. This would be expected, as the length of the growing season largely determines the amount of heat and light available for plant growth. This should have as much influence on varietal characters as the distance from the equator, because of differences in altitude and other conditions. For example, Figure 1 shows that Crossville, Ala., and Florence, S. C., are located at approximately the same latitude; yet the annual average number of frost-free days at Crossville is approximately 35 days less than at Florence.

In interpreting the data presented in Table 1 in which comparisons are made between the yield of local and introduced cottonseed, it should be remembered that the local seed of unnamed varieties were in most instances badly mixed and considered undesirable for planting purposes. The introduced seed were of so-called purebred varieties from the best cotton breeders. While the average difference in yield in favor of home-grown seed is not statistically significant, it certainly suggests that there is an advantage in growing cotton from seed produced under local conditions.

The importance of natural selection in cotton breeding, and the part that local climatic conditions play in the acclimatization and adaptation of a local or introduced cotton variety, are probably not fully appreciated by many who think the methods and skill of cotton breeders largely determine their success in the breeding of locally and widely adapted cotton varieties. A correlation study (6, p. 10) showed no significant relationship between the yield of cotton plant

selections and their progeny, although most of the present cotton varieties were developed by the plant-to-row method.

Angleton, Tex., approximate latitude  $29^{\circ} 15'$ , located in the extreme southern edge of the Cotton Belt, has an average length of day of about 14.1 hours on June 21 (4, p. 10) while Wyatt, Mo., with an approximate latitude of  $36^{\circ} 45'$ , located in the extreme northern edge, has about 14.8 hours of daylight on the same date. The difference in the length of day and the total amount of light available for plant use at the various latitudes included in this study is not great, but as the cotton plant is very sensitive to low temperatures, there is a difference of about two months in planting dates at the southern and northern edges of this territory.

The cotton plant is sensitive to sunlight and does not fruit well without an abundance of it. The percentage of the total possible amount of sunshine (9) during the growing season for the Cotton Belt varies more with changes in longitude than with latitude. Oklahoma and west Texas get from 70 to 80 per cent while much of Georgia and Alabama get only 50 to 55 per cent of the total or possible amount of sunshine during the summer. None of the Texas varieties tested east of that State made high yields. It is likely that differences in amount of sunshine in the eastern and western parts of the Cotton Belt account in part for the poor adaptation of western cottons to eastern conditions.

The amount of rainfall for the Cotton Belt (5, p. 26-27) also varies more with changes in longitude than with latitude, but not so much as does the amount of sunshine. The eastern part of the Cotton Belt and much of the territory along the Gulf coast has an average summer precipitation of from 14 to 19 inches, while most of the central and western parts get only about 8 to 14 inches of rainfall during the three summer months.

Another variable in growing conditions at different locations in the Cotton Belt is the infestation and damage by the cotton boll weevil (*Anthonomus grandis*). The length of growing season as measured by the number of frost-free days may be much greater in southern latitudes and at low altitudes than in northern latitudes and at high altitudes, but because of the nature of boll-weevil activities the cotton-fruiting period is often about the same length of time. Much of the northern part of the Cotton Belt is free, or practically so, of boll-weevil damage, while the southern half and most of the Atlantic Coastal Plain is infested with boll weevils every year. Boll-weevil infestation tends to shorten the normal length of fruiting period by preventing cotton from setting and maturing bolls during the latter part of its normal fruiting period. Varieties bred for short growing seasons in northern latitudes may sometimes be better adapted to southern latitudes than are varieties bred for long growing periods, especially during seasons favorable to the boll weevil.

The differences in climatic, boll-weevil, and soil conditions at different locations in the Cotton Belt may explain, at least in part, the observed tendency for locally grown seed to outyield seed from distant places.

#### SUMMARY

The approximate latitude and number of frost-free days was determined for 37 locations where cotton varietal tests have been conducted for a period of at least three years. The same data, the relative yield,

the average annual yield, and the place of origin of seed was determined for the varieties tested at each. A correlation coefficient of  $-0.143$  for relative yield and differences in latitude at the test location and place of origin was found. A coefficient of  $-0.118$  for relative yield and differences in number of frost-free days at test location and place of origin was found.

Information on the comparative yielding ability of local and introduced cottonseed in 68 experiments located in four States is given. Locally grown seed outyielded introduced seed in 60.3 per cent of the places where the average yield of all named varieties was compared with the yield of local seed.

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# THE HAIRY-VETCH BRUCHID, *BRUCHUS BRACHIALIS* FAHRAEUS, IN THE UNITED STATES<sup>1</sup>

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## INTRODUCTION

The genus *Bruchus* was restricted by Schilsky (6)<sup>2</sup> to include only the immediate allies of *Bruchus pisorum* (L.), the pea weevil, of which he tabulated 24 species known to him. To these may be added others doubtfully distinct, imperfectly known, or more recently described, which increase the nominal species of the genus to a total of about 46. All these species are native to the Palearctic region. Two of them are already well known in the United States as major pests of the plants affected. *B. pisorum* was the first of the genus and one of the first species of the family to be recognized. It was described in 1752, and was recorded as having destroyed in the 1740's the flourishing colonial American industry of producing dry peas for ships' stores. *B. rufimanus* Boheman, the broadbean weevil, has now practically destroyed the broadbean industry of California. A third species, *B. brachialis* Fahraeus, has in recent years gained a foothold in this country, for in 1931 the junior author found it heavily infesting the seeds of vetches growing in New Jersey, Delaware, Maryland, and North Carolina, and in 1932 it was found in Virginia. In view of these facts it seems wise to present a brief summary of the knowledge at present available of the habits of the members of this genus and to point out the increased danger of their establishment in the United States as a result of changed commercial conditions.

## FOOD PLANTS OF *BRUCHUS* (RESTRICTED)<sup>3</sup>

All species of *Bruchus* of which we have credible records<sup>4</sup> feed as larvae in seeds of plants of the leguminous tribe Viciaeae, infesting the pods in the field before they ripen. They are unable to reinfest the seed in storage.

<sup>1</sup> Received for publication June 30, 1932; issued May, 1933.

<sup>2</sup> Reference is made by number (italics) to Literature Cited, p. 750.

<sup>3</sup> Unless otherwise indicated, the records of host plants of the species herein discussed are all based upon determinations, made by the senior author of insects associated with their host seeds contained in the collections of the U. S. National Museum. A large part of this material was obtained in routine inspection work by officers of the Federal Horticultural Board and its successor, the Plant Quarantine and Control Administration (now the Bureau of Plant Quarantine), of the U. S. Department of Agriculture. Much of the seed material was imported by the Division of Foreign Plant Introduction for the experimental work of the Bureau of Plant Industry. The determination of the seeds has been verified by Roland McKee, in charge of investigations of the vetchlike plants, or by H. C. Skeels, in charge of the seed collection of the Division of Foreign Plant Introduction. To both of these men the writers are indebted for assistance in botanical and agricultural matters in the preparation of the present work.

<sup>4</sup> Records of species affecting the seeds of *Lotus*, *Ulex*, *Phaseolus*, *Oxytropis*, and *Lupinus* are not considered credible. They seem to be based upon visits of adult bruchids to flowers or extrafloral nectaries, upon erroneous identification of seeds affected, or upon mistaken interpretations of the literature.

The tribe Viciae includes the genera *Vicia*, *Lathyrus*, *Lentilla* (or *Lens*), *Pisum*, *Cicer*, and *Abrus*. *Cicer* (which includes *C. arietinum* L., the chickpea, garbanzo, or gram) and *Abrus* (including *A. precatorius* L., the rosary-pea) are known to be infested by other Bruchidae, but no species of *Bruchus* has yet been recorded as affecting either genus. The other host genera all have some species affected; in some instances two or more genera may be attacked by one species of *Bruchus*.<sup>5</sup> These genera are very closely allied and merge into one another imperceptibly, particularly in the characters of seeds and pods, so that their separation is more or less conventional, being based on usage rather than on sound botanical differences. The seeds of *Vicia*, *Lathyrus*, and *Pisum* are often not easily distinguished as to species, or even as to genus, by botanists familiar with the plants. Hence, many records of host plants of species of *Bruchus* are indefinite from lack of specific determination of the plants affected or erroneous from misdetermination of the plant. Furthermore, the species of *Bruchus* are difficult to recognize and frequently have been determined wrongly. The records of attacks upon definite species of Viciae are surprisingly few, and these few are less to be depended upon than is desirable.

*Lentilla lens* (L.) W. F. Wight (*Lens esculenta* Moench), the lentil, is affected by *Bruchus lentis* Froelich, *B. ervi* Froelich, *B. signaticornis* Gyllenhal (*pallidicornis* Boheman), and *B. rufimanus* Boheman. The first two species are not known to affect other host plants. *B. signaticornis* is also found in the lentil-like seeds of *Vicia monanthos* Desfontaines. *B. rufimanus* seems to attack lentils only incidentally. The cultivation of lentils has been repeatedly abandoned in various parts of Europe on account of the attacks of one or another of the lentil bruchids. The writers have found no records of bruchids attacking the seeds of other species of *Lentilla* than those mentioned.

*Pisum sativum* L., the common pea with its varieties, is attacked by *Bruchus pisorum* (L.), *B. emarginatus* Allard, *B. tristiculus* Fahraeus, and perhaps by *B. tristis* Boheman. *B. emarginatus* seems to be the species that attacks the pea in India which has sometimes been determined as *B. affinis* Froelich. It is evidently quite as much a pea pest as the better known *B. pisorum*. *B. tristiculus* is more often found in seeds of *Lathyrus*. The writers have not been able to verify the records of *B. tristis* attacking the pea, and it is possible that they may be based on misdeterminations of *B. tristiculus*. Peyerimhoff (10, p. 365) has reported the pea weevil breeding in seeds of *P. elatius* Bieberstein, the reputed ancestor of the pea, in northern Africa, and the writers have found it in this species from Tiflis. The writers have discovered no records of the other species of *Pisum* being attacked by Bruchidae.

The species of the genus *Lathyrus* are not very familiar to laymen in the United States except *L. odoratus* L., the sweet pea. There is a considerable industry in the production of the seed of this species in California which is definitely threatened by the likelihood that *Bruchus affinis*, *B. tristis*, *B. tristiculus*, and *B. rufipes* Herbst<sup>6</sup> will

<sup>5</sup> It should not be inferred that species of *Bruchus* are the only Bruchidae affecting plants of the other genera of the tribe Viciae. Certain species of Bruchidius and Callosobruchus affect a number of them. Some species of Bruchidius may be as definitely attached to some of them as the pea weevil is to the pea.

<sup>6</sup> Since this paragraph was written *Bruchus rufipes* has been intercepted by inspectors of the Bureau of Plant Quarantine in seeds of sweet pea (*Lathyrus odoratus*), common vetch (*Vicia sativa*), and hairy vetch (*V. villosa*), all of European origin.

be introduced in sweet peas or the seeds of other species of *Lathyrus*, particularly *L. sativus* L.<sup>6</sup> The grass pea (*L. sativus*) is an important food grain in the south of Europe and in India and is also imported into the United States for use as food. The Tangier pea (*L. tingitanus* L.) has been grown experimentally as a forage and green-manure plant, but it has not yet made a place for itself in our agriculture. Other species have also been grown as ornamentals, and still others experimentally as forage crops. *B. venustus* Fahraeus, *B. viciae* Olivier, *B. loti* Paykull, *B. rufipes*, *B. tristis*, *B. tristiculus*, *B. affinis*, and *B. atomarius* (L.) are more or less definitely known to breed in seeds of some species of *Lathyrus*. *B. altaicus* Fahraeus was described from the seeds of *L. tuberosus* L. from the Altai Mountains in central Asia, and the writers have recently determined as this species a bruchid found in the seeds of an undetermined species of *Vicia* from Turkestan.

Several species of *Vicia* are used for forage, hay, and green-manure plants, and the seeds of some of them are occasionally used for human food. *Vicia faba* L. (the broadbean) is a common food of man. *Bruchus rufimanus* seems to prefer *V. faba* as host and is principally known as an enemy of that plant. Peyerimhoff (10, p. 365) has recorded it as bred from *V. vestita* Boissier, which is a synonym of *V. lutea* L., in which the present writers have also found it breeding. The writers' notes also record it from *V. monanthos* Desfontaines, *V. sativa leucosperma* Moench, *V. narbonensis* L., and *Lentilla lens*. *B. dentipes* (Baudi) is reported from *V. faba*, and the writers have determined it from a wild *Vicia* from Haifa, Palestine. Bekman (4) records *B. dentipes* Baudi from *V. hyrcana* (= *hyrcanica* Fischer and Meyer). As before indicated, the writers have determined *B. signaticornis* from seeds of *V. monanthos* from Madrid, Spain. *B. viciae* Olivier, *B. atomarius* (L.), *B. ulicis* Mulsant and Rey, *B. rufipes* Herbst, *B. griseomaculatus* Gyllenhal, and *B. brachialis* Fahraeus are all recorded as breeding in seeds of species of *Vicia*.

#### COMMERCIAL CONDITIONS AFFECTING THE DISTRIBUTION OF BRUCHIDAE

The establishment of *Bruchus brachialis* in the eastern part of the United States is the result of a post-war speeding up of agricultural commerce which has increased the chances of introducing exotic Bruchidae into this country. Formerly commercial operations were so slow that seeds were not distributed in this country until a year or more after they were harvested. The bruchids in them therefore had time to emerge and if they were unable to reinfest the seed they died before they were scattered over the country. Within the last 20 years the principal shipping points of hairy-vetch seed imported into the United States have been changed from Baltic to Adriatic ports, where the earlier season makes it possible for the seed to be ready for market in the same season in which it is harvested. It is also much more certainly infested by *B. brachialis* in southern Europe than in the more northerly regions.

During the last three years several lots of living bruchids have been noticed in seeds imported under regular trade conditions, whereas in previous consignments the insects in the seeds examined were dead. *Bruchus brachialis* is the seventh species of Bruchidae known to have been accidentally established in continental United States.

<sup>6</sup> See note on page 740.

## HISTORY OF BRUCHUS BRACHIALIS

*Bruchus brachialis* was described by Fahraeus (13, p. 79) from specimens sent to Schoenherr for description by Chevrolat, the material coming from Tours, France.

Mulsant and Rey (8, p. 33) described the male of *brachialis* as *pallidicornis* Schoenherr, and gave the first description of its distinctive sexual characters.

Allard (1) renamed *pallidicornis* Mulsant and Rey (not Gyllenhal) as *ruficornis*, and added Sicily to France as its habitat.

Perris (9, p. 237) records that in September, 1874:

I fanned some wheat \* \* \*. Under the fan the seeds of vetch and the *Bruchus* from them had accumulated in such numbers as to cover the ground. I noticed many *Bruchus pisi* [pisorum] and *granarius* [atomarius] with which I was not concerned, and persuaded myself the rest were *nubilus* [rufipes]. Entirely by chance, I picked up a few of them and saw that *nubilus* was in a very small minority and that the rest belonged to one or two species unfamiliar to me, though I might have mistaken them for *sertatus* [ervi] or *pallidicornis* [signaticornis], if I did not know that those species live in lentils. To have some for study when not too much pressed for time I collected some hundreds of them and found the two species to be *brachialis* Fahr. and *ruficornis* All., which according to Mr. Allard himself is only the male of *brachialis*.

Here then is an insect which, judged by catalogues of rich collections, which I had run through recently, my colleagues do not have, which I did not myself possess, and of which, doubtless, I may take thousands more, for there are always, to nourish them, enough vetches among the wheat.

Baudi (2, p. 13, 16) described the species, placed it in Mylabris, and extended its range to include "all Italy and its Islands, Dalmatia, France, Spain, and Algeria."

Bedel (3, p. 357) placed it in Laria, gave its range in France as south of the Loire River, and added the record of its occurrence in Asia Minor.

Marchal (7) gives an account so pertinent to the present occasion that it is quoted in its entirety. Now, after nearly 30 years, his prediction of its establishment in this country has been fulfilled. The account is as follows:

Perris has already indicated the presence of *Laria brachialis* Fahraeus in the seeds of vetches growing in fields of wheat. He found it there in quantity, associated with some other species, in seeds of *Vicia* accumulated under a fan which had just been used for cleaning wheat. The specific name of the plant was not definitely indicated by the author.

Examination of a package received through Mr. Hariot, preparator at the Museum, results in finding this insect attacking the seeds of vetches cultivated as forage plants and notably those of *Vicia villosa*. The seeds of this plant sent to me were strongly infested by *Laria brachialis*, each seed attacked containing one of these insects.

It should be noted that *Vicia villosa* is a plant of northern, eastern, and central Europe and does not grow wild in France. It has, however, for some years been cultivated frequently as a forage plant. On the other hand, *Laria brachialis* is a species belonging in the south of Europe and [according to Bedel] does not reach above the Loire in France except accidentally. Apparently, then, it is only recently that this insect has adapted itself to *Vicia villosa*. [This inference may be doubted.]

Not only in France is this plant menaced by this insect, which like all bruchids attacking plants under cultivation doubtless will not delay becoming cosmopolitan. The seeds sent to me had, indeed, been supplied by a French seed house and forwarded to Canada, whence they had been returned to France for examination of the insects contained in them. The approaching naturalization of *Laria brachialis* in America is an event to be foreseen.

Schilsky (6, No. 29) placed the species in *Bruchus* as restricted by himself and gave the most recent and most careful description of the species, a translation of which follows:

*Bruchus brachialis* Fahraeus. Ovale, black, covered with sparse fuscous pubescence beneath, sides of breast and venter whitish-maculate; pronotum and elytra whitish-variegate; pygidium covered with whitish-cinereous pubescence; prothorax almost twice as broad as long, sides parallel behind, dentate in the middle, hind angles rectangular; elytra short; front legs rufotestaceous, hind femora strongly dentate, hind tibiae ending in two spines at apex beneath. Length, 3-3.5 mm.

Male. Antennae rufotestaceous, joints 6 to 10 depressed, strongly transverse; front tibiae stout, compressed, slightly arcuate; middle tibiae curved, armed at apex with a truncate mucro.

Female. Antennae a little less depressed, black, first four or five joints and the last (and the front legs also) rufotestaceous; front femora black at base; front tibiae linear.

Variety a [male] with antennae infusate in the middle; variety b [male], front femora black; variety c [female], front femora and last joint of antennae black; variety d [female], two joints of antennae rufous or ferruginous; variety e [female], antennae rufotestaceous, joints 6-7 or 6-9 infusate; variety f [female], antennae bright rufotestaceous (Erivan); variety g [female], last joint of antennae black, middle tarsi rufescent. Nyons (from Baudi).

[Bibliography omitted.]

Antennae differing in color in the sexes as in *Br. pallidicornis*; front tibiae of male in both species widened but the fasciate markings of elytra lacking [in *brachialis*]; middle tibiae and tarsi black, the middle tibiae of males have very different characters. Body black, oval, similar to *atomarius*, sparse very short pubescence beneath, sides of breast and sternites with usual brighter maculae, brownish and dirty white variegate above, indefinite white markings of elytra not distinctly transverse-fasciate; scutellum and a macula in front of it whitish pubescent; pygidium evenly cinereous, generally with two small indistinct spots; pronotum almost twice as broad as long, densely rugosely punctured, the sides parallel behind, a small denticle in the middle, hind angles right angles, sides not emarginate in front of the angles; elytra short, oval, the striae fine, almost impunctate; hind femora strongly toothed, hind tibiae within with two equal very short spinules at apex.

Male. Antennae yellow, rarely infusate in the middle (variety a), compressed, joints 2 to 4 fully as long as broad, or shorter, 5 considerably broader, the following joints very strongly transverse, emarginate at apex; front legs reddish yellow, their tibiae broad, compressed, parallel-sided, hence not arcuately expanded on the outer side [as it is in *signaticornis*, the other common species with broad front tibiae in the male]; middle tibiae curved downward, with a black, truncate plate at apex directed backward. In one male from Dalmatia (von Heyden collection) the middle tarsi are red.

Female. Antennae narrower, black; the 4 or 5 basal joints and the last, rarely the last two (variety d), reddish yellow; rarely [are] the antennae reddish yellow infusate in the middle (variety e); base of the yellow front femora generally black to the middle, rarely entirely black (variety b); in some cases the last joint of the antennae is also black (variety c).

In central and southern Europe, in Asia Minor (Erivan, Anatolia), in Algeria.

#### SYNOPTIC TABLE OF THE NEAREST ALLIES OF BRUCHUS BRACHIALIS

According to Schilsky (6, No. 12), *Bruchus hamatus* Miller (12, p. 228) from Greece and Asia Minor is very closely allied to *B. brachialis*, but the pronotum is much less transverse and the pale dots on the elytra are arranged in two indistinct fasciae. The male *hamatus* has the secondary sexual characters of *brachialis*, but the fifth joint of the antennae is produced into a tooth or spine such that if the following joints were modified in the same manner the antennae would be pectinate. This is a rare species and its food plants are unknown.

*Bruchus venustus* Fahraeus (13, p. 75) is also closely allied to *brachialis*, but it has the hind femora with a small subbasal denticle beneath instead of the usual subapical tooth. It is found in the Caucasus, Hungary, and southern France and is considered a rare species.

*Bruchus terminatus* Wollaston (14, p. 381) from Teneriffe is apparently near *brachialis*, but it is more elongate and has more pale pubescence. The description of the subbifid spine at the apex of the middle tibiae would seem to refer to a truncate lamella such as occurs in *brachialis*, *venustus*, *ulicis*, *hamatus*, and *signaticornis*. The following outline should differentiate the males of this group:

1. Middle tibiae with acute tooth above apical lamella, front tibiae incrassate, antennae usually pale.....*signaticornis*
2. Middle tibiae without tooth above apical lamella..... 2
2. Front tibiae incrassate..... 3
3. Front tibiae simple, antennae dark except at base.....*ulicis*
3. Antennae pale, joint 5 produced into a tooth, middle femora triangularly widened, front legs only pale.....*hamatus*
- Joint 5 of antennae normal..... 4
4. Hind femora with small denticle near base, without subapical tooth, front legs only pale, antennae with joints 1 to 8 pale, 9 to 11 dark.....*venustus*
- Hind femora normal, with subapical tooth..... 5
5. Antennae pale, front legs only pale.....*brachialis*
- Antennae dark, front legs and middle tarsi pale.....*terminatus*

It is not possible at present to differentiate the females of these species or either sex of some other species which are as yet imperfectly known.

The hairy-vetch bruchid may be distinguished readily from the other two species of *Bruchus* now known to be established in the United States by its smaller size, much more transverse pronotum, greatly reduced pale pubescence, particularly on the elytra, and the coloration of the antennae, pale in the male, basal joints and terminal joint pale in the female. These three species may be distinguished from other North American Bruchidae by the emarginate lateral margin of the pronotum with a small distinct tooth before the emargination.

#### BRUCHUS BRACHIALIS IN THE NATIONAL MUSEUM

*Bruchus brachialis* has been recorded from no host plant other than *Vicia villosa* Roth. In addition to recently collected material from the eastern part of the United States, it is represented in the National Museum by three lots of adults with which are preserved seeds of *V. villosa* from which they had emerged. The first is from the Chittenden collection and was secured from the French exhibit in the Paris Exposition of 1889. The second was secured by the senior author in December, 1921, from seeds of *V. villosa* imported by a seed firm in Baltimore. Finding the seeds infested, this firm had brought the matter to the attention of the United States Department of Agriculture. The beetles contained in this lot were all dead when examined. The seeds were very lightly infested, much less than 1 per cent showing the work of *B. brachialis*. The third lot was submitted to the senior writer in September, 1930, by the manager of a seed-testing laboratory in Washington, D. C. The insects had been found living in samples of *V. villosa* seed submitted for testing by a Hungarian seed firm which proposed marketing the seeds in the United States. These

samples also showed less than 1 per cent infestation. After all the emerged bruchids had been removed, the seeds were kept under rigorous quarantine conditions at room temperature through the following winter, but no additional adults emerged. This suggests that at room temperature all the larvae transform and emerge in the fall or die before spring.

#### LIFE HISTORY OF BRUCHUS BRACHIALIS UNDER CONDITIONS PREVAILING IN NEW JERSEY

Adults of *Bruchus brachialis* were taken by sweeping in a fallow field near Haddon Heights, N. J., on June 12, 1931. On June 17, this field was again visited and many adults were collected. At this time eggs were being deposited in large numbers on the pods of the hairy vetch. Some preliminary observations made by the junior writer on the habits of the insect in this and in other patches of vetch are given here.

##### THE EGG

The egg is about 0.6 mm long, 0.2 mm wide at the widest point, which is near the anterior end, and 0.2 mm high. Viewed from above, it is more or less oblong in outline, with the ends rounded and the sides straight, or nearly so, and converging somewhat toward the posterior end. The outline is similar when viewed from the side, and from the end it appears nearly circular. The egg is glued lengthwise to the outer surface of the pod. The exposed surface is finely wrinkled.

The freshly deposited egg is a pale yellowish green. The hatched egg appears whitish or straw colored, owing to the debris with which the shell is filled by the larva in gaining entrance into the pod. In the field many of the eggs darken.

Eggs are deposited on the immature pod after it has attained a width of about one-fourth inch. Most of the eggs are placed near and parallel to the margins of the pod, with the posterior ends directed toward the apex of the latter. Twenty-five or more eggs were seen attached to a single pod, but the usual number was between 3 and 10. With the exception of those developed from late flowers, few pods escaped attack.

##### THE LARVA

The young larva gains entrance to its host by gnawing a hole through the broader end of the chorion of the egg and through the thin valve of the legume to which it is attached. It then turns toward the center of the pod in search of a seed. A few larvae do not find the seeds and therefore die within the pod. The larva may enter the seed at any point, the hilum being often preferred. In the ripe seed the entrance hole of the larva may be seen under low magnification as a small circular hole filled with frass. As many as seven entrance holes have been noted in a single seed, but rarely does more than one larva develop.

Once inside the seed, the larva develops rapidly, feeding upon the seed content. During growth the body of the larva is surrounded by dry frass, and when full grown it packs this loose material against the sides of the cavity made in feeding, to form the oval pupal chamber in which it is to transform. The frass is held in place by a thin cement substance, which upon drying hardens into a fibrous cocoon

very resistant to solvents. The full-grown larva often narrowly thins the margin of the circular area of the outer seed coat which forms the cephalic end of the cell. From the outside this thinned area appears as a narrow ring of pale olive green and is rather conspicuous against the darker color of the seed. The ring marks the area or "cap" which the adult cuts out in emerging. The middle of the cap is often thinned, giving it the lighter color.

The full-grown larva is a distinct yellow in color, and in outline resembles the larva of the common bean weevil (*Mylabris*) *Acanthoscelides obtectus* (Say) (5). After the larva has finished feeding and has the inside of the cap properly thinned and the cell lined, it is ready to pupate. Before pupating, however, it passes through a prepupal stage during which the shape of the pupa is gradually assumed, the body becoming less curved with a distinct constriction at the neck. This change takes place in about two days.

#### THE PUPA

The pupal stage lasts from 5 to 5½ days. During the first 3 days there is no change in the pale yellow color except in the eyes. Soon after pupation minute reddish spots appear along the hind margin of the eye. As these spots enlarge and darken, others gradually appear toward the front of the eye, the apex of the upper lobe being the last area to show the coloring. Each spot occupies the center of a facet, the interspaces being the pale body color until the fourth day, when the eye has become a uniform dark reddish brown. About this time a curved brownish line inside the hind coxal cavity appears and the mandibles start to become brown at the apex. The fifth day brings considerable change in color, the tarsal claws becoming fuscous at the base, and the body, except the abdomen, becoming various shades of pale reddish brown, the color being darkest at the articulations of the legs.

#### THE ADULT

The adult at first resembles the last stage of the pupa, but in less than 12 hours it has become normal in color. The wings remain exposed beyond the apex of the elytra for a day or two. The abdomen is much distended at first and about four days are required for it to contract to normal form. The adult is then thoroughly hardened and quite active.

Soon after it is fully hardened the adult cuts around the circular cap, pushes it away, and leaves the seed. The exit hole is located opposite to or near one end of the seed scar, but does not include any part of the scar. The first emergence from caged material collected June 17 took place about July 15, and during the rest of July adults emerged in enormous numbers from all collections. In the field on July 22 many emergences had already taken place and adults were numerous in unopened pods. The adult is unable to cut its way through the valve of the pod and depends upon the dehiscence of the pod for its escape.

Late pods in condition for oviposition on July 22 were without eggs and no recently deposited eggs were seen on any pods, indicating that the egg-laying season of the overwintering females had been ended for some time and that the newly emerged females were not depositing eggs.



The life history of *Bruchus brachialis*, therefore, may be briefly stated as follows: The overwintering females deposit eggs on immature pods of vetch in June, and the new generation of adults emerges from the seeds during the latter half of July and the first part of August but does not deposit eggs until the following June. It has not been ascertained that any individuals overwinter in the seeds.

#### QUANTITY OF FOOD CONSUMED AND EXTENT OF INFESTATION

As the larva does not migrate from one seed to another, the quantity of food consumed depends largely upon the size of the seed. This often directly affects the size of the adult, for small, poorly developed seeds produced adults 2 mm in length while the normal individual is 3 to 3.5 mm long. The entire content of the smaller seed is eaten, but the larger seed contains more food than is required by the larva. It is unlikely that a seed in which a larva has developed would be able to germinate.

A small representative sample of hairy-vetch seed from the first pods to ripen at Haddon Heights, N. J., was examined and the infestation noted. Of 785 seeds, 15 were not at all developed and are not considered. Of the remaining 770 seeds, 591 (76.7 per cent) produced adults or had pupae or full-grown larvae when opened, 32 (4.2 per cent) contained larvae that had died soon after entering the seed, and 147 (19.1 per cent) were not infested. This gives a total infestation of 80.9 per cent. One hundred of the uninfested seeds weighed 2.1 grams. Thus 1 pound would contain about 21,600 seeds, which would theoretically produce more than 17,000 bruchids.

#### PARASITES

The common bruchid egg parasite, *Uscana semifumipennis* Girault, was not noted to attack the eggs of *Bruchus brachialis*, but it should be expected to do so.

Six species of native American Chalcidoidea usually affecting coleopterous larvae and pupae in similar habitats were found parasitizing *Bruchus brachialis* in *Vicia villosa* at Haddon Heights late in July and in August, 1931. These species, kindly determined for the writers by A. B. Gahan, of the Bureau of Entomology, are *Eupelmus cyaniceps amicus* Gir., *Eupelminus saltator* (Lindemann), *Microdonotomerus anthonomi* (Crawford), *Zatropis incertus* (Ashm.), *Habrocytus* sp., and *Eurytoma tylodermatis* Ashm. These species, except *E. saltator*, have been previously recorded as enemies of Bruchidae, but, as suggested, they are not in any peculiar sense bruchid parasites.

#### PRESENT DISTRIBUTION IN THE UNITED STATES

*Bruchus brachialis* is thus far known from five States—New Jersey, Delaware, Maryland, North Carolina, and Virginia. Numerous infestations in New Jersey were found, involving Camden, Burlington, Atlantic, and Cape May Counties, all in the southern part of the State. The junior writer reared numbers of the bruchid from vetch pods collected by D. P. Perry at Felton, Kent County, Del., July 1, 1931, and at Salisbury, Wicomico County, on the Eastern Shore of Maryland July 15, 1931. In September, 1931, a small sample of vetch seed was received at Haddon Heights, N. J., for experimental planting from

Roland McKee, of the Bureau of Plant Industry, United States Department of Agriculture. This lot of seed originated in the vicinity of Woodleaf, Rowan County, in the western part of North Carolina, where the smooth variety has been grown for several years. Several dead adults of *B. brachialis* were taken from seeds in this lot and a number of seeds with emergence holes were also obtained, showing that the insect is established in that State. During June, 1932, the junior author found this species in additional localities in Maryland, in the District of Columbia, and in adjacent Virginia.<sup>7</sup>

#### HOST PLANTS OF BRUCHUS BRACHIALIS

*Vicia villosa* is the only previously recorded host of *Bruchus brachialis* and is the species involved in the foregoing records. At Haddon Heights, Camden County, N. J., *V. cracca* L. was found to be lightly infested with this species of *Bruchus*. Normal emergence from the seeds was obtained during the first part of August from material collected July 11 and 23, 1931. The habits of the bruchid in these hosts, so far as known, are the same as in *V. villosa*.

During 1932 the junior author found *Bruchus brachialis* attacking the woolly-pot vetch (*Vicia dasycarpa* Ten.) and the Hungarian vetch (*V. pannonica* Crantz) in experimental plantings. *Vicia angustifolia* Roth growing abundantly in a field with *V. villosa* was not found to be infested.

#### ECONOMIC RELATIONS OF BRUCHUS BRACHIALIS

The hairy vetch (*Vicia villosa*) is an exceedingly adaptable forage and green-manure plant highly recommended by the United States Department of Agriculture for cultivation in this country in sections not favorable for the cultivation of alfalfa, red clover, or crimson clover. Its cultivation has been gradually increasing, particularly on the Atlantic seaboard from Washington south. The planting requirements in recent years have called for about 2,000,000 pounds of seed, of which about one half has been produced in this country and the remainder imported from Europe.

*Vicia cracca* is a circumpolar species extending from Europe across northern Asia and America to the Northeastern States, growing wild in sections north of those adapted to hairy vetch. It has not been found sufficiently useful in cultivation to compete with red clover and is of little commercial importance although it has most of the good qualities of hairy vetch. There is no production of its seed for commercial purposes. Hungarian vetch (*V. pannonica*) serves the same uses as the hairy vetch and the common vetch and recently has partly

<sup>7</sup> Since this paper was submitted for publication, notes have been received from G. W. Underhill, associate entomologist of the Virginia Agricultural Experiment Station, on the oviposition of *Bruchus brachialis* on the pods of hairy vetch near Richmond, Va., in 1931 and 1932. On 40 pods examined in late July, 1931, an average of 26 eggs per pod were found, with a maximum of 48 and a minimum of 11. The first emergence from the seed was noted on July 27, and a weevil emerged from almost every seed. Freshly laid eggs were observed on May 23, 1932, and were abundant in early June. Larvae hatched from the eggs of May 23 on May 27 and 28. In neither year were adults observed to cut their way out of the pods. A vetch not identified by Underhill, but certainly *Vicia angustifolia*, was abundantly associated with *V. villosa* but was not attacked by *B. brachialis*. Underhill believes that he found *B. brachialis* in small numbers in 1930 at Richmond, and the junior author certainly did at Haddon Heights, N. J. It is probable that the infestations in both places are recent. In New Jersey, at Richmond, Va., and in North Carolina *B. brachialis* was noticeably more abundant in 1932 than in 1931. Underhill's notes indicate that the season for *B. brachialis* at Richmond is three weeks or more earlier than at Haddon Heights. A thorough survey of the eastern part of the United States may show the bruchid to be established elsewhere.

superseded *V. sativa* in the vetch-growing sections of Oregon, where there is now a considerable commercial production of its seed.

The woolly-pod vetch (*Vicia dasycarpa*) seems to serve the same uses, but has not become as popular as some of the other vetches, and there is little or no commercial production of its seed in this country. *Bruchus brachialis* is important only in its relation to the production of the seeds of the vetches attacked by it. To what extent it will affect the production of hairy-vetch seed in Oregon, Indiana, Michigan, Ohio, New York, and the Carolinas, and of Hungarian-vetch seed in Oregon can not be predicted, but the North Carolina growers are already aware of increasing losses from it.

Whether the present species will also affect the common vetch (*Vicia sativa*) is entirely problematic. While no records have been encountered of attacks on other species of vetch, this must be considered of little significance until comprehensive studies have been made on the biology of this and other species of *Bruchus*. Several other species of *Bruchus*, however, attack *V. sativa* in Europe.

#### TWO OTHER SPECIES OF BRUCHUS FROM VETCHES

The bitter vetch (*Vicia ervilia* (L.) Willdenow) is another green-manure plant from the Old World occasionally cultivated experimentally in the United States, but it is still uncertain whether it will find an important place in American agriculture. Under certain commercial conditions the seeds of this species are imported and ground for mixed poultry feeds. The National Museum contains about 10 lots of *Bruchus ulicis* with notes indicating that the beetles were bred from seed of *V. ervilia* of European origin. Whether this insect affects other species of vetches is not a matter of record.

A sample of the seed of *Vicia cracca* introduced from Japan under the name *V. gemella* Crantz by the Office of Foreign Plant Introduction in 1903 was recently examined and a single female *Bruchus* found in it which agrees with the description of *Bruchus maculatipes* Pic (11). This vetch is so similar to *V. villosa* that it is almost certain that this species would be able to utilize the hairy vetch.

In May, 1932, individuals of the same species were sent by S. I. Kuwana to the Bureau of Entomology for determination, having been taken from among seeds in storage at Kyoto, Japan. Two males among these make it possible to recognize its close relationship with *Bruchus rufipes*, the main difference in *B. maculatipes* consisting in the almost complete suppression of the pale pubescent pattern of the pronotum and elytra.

#### SUMMARY

The hairy-vetch bruchid (*Bruchus brachialis* Fahraeus) is now established in the United States. In 1932 it was known to occur in New Jersey, Delaware, Maryland, the District of Columbia, Virginia, and North Carolina. It affects the seeds of the cultivated forage plants hairy vetch, woolly-pod vetch, Hungarian vetch, and the wild *Vicia cracca*. It is of economic significance because it destroys the seeds of its host plants.

The insect belongs to the restricted genus *Bruchus*. All the members of this genus deposit their eggs upon the green pods of the host plants. Their larvae feed within the seeds and transform there.

The adults emerge from the ripe seeds and can not reinfest them. *Bruchus brachialis* seems always to transform and leave the seeds in summer and hibernate elsewhere as an adult.

The adult is described and characters are pointed out to distinguish *Bruchus brachialis* from the closely allied European species of *Bruchus* and from all other American Bruchidae.

Notes on the life history and habits of *Bruchus brachialis* in New Jersey are recorded. In Haddon Heights, N. J., about 80 per cent of the seeds of volunteer plants of the hairy vetch produced during the egg-laying period of the bruchid were destroyed by it. Six species of native American Chalcidoidea were found parasitizing it.

The incidence of the different species of *Bruchus* upon seeds of the leguminous tribe Viciaeae, including numerous important economic plants, is summarized, the records being based mainly upon interceptions made in the course of the work of the Department of Agriculture. Changed commercial conditions in handling these seeds have led to the establishment of *Bruchus brachialis* and make probable the establishment in the United States of other destructive species affecting plants upon which agricultural industries are based.

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# THE ASSOCIATIVE EFFECTS OF FEEDS IN RELATION TO THE UTILIZATION OF FEED ENERGY<sup>1</sup>

By E. B. FORBES, *Director*, WINFRED W. BRAMAN and MAX KRISS, *Associate Professors of Animal Nutrition*, and R. W. SWIFT, *Associate in Animal Nutrition*, with the collaboration of ALEX BLACK, DONALD E. FREAR, O. J. KAHLBERG, F. J. McCLURE, and LEROY VORIS, *Institute of Animal Nutrition, Pennsylvania State College*

## INTRODUCTION

The question of methods of measurement of the net energy of single feeding stuffs and of rations has been before this Institute, in one form or another, for many years, either as such values have been directly sought, or as efforts have been made to analyze the problem and to elucidate the underlying principles involved.

In connection with the direct approach to this subject consideration has been given to various methods of determining net energy (11)<sup>2</sup> and to a new method of applying the net energy conception in the derivation of a feeding standard for milk production (14). In the analysis of the fundamental aspect of this problem studies have been made on the influence of type and age on the utilization of feed (2); on the influence of position, as to standing or lying, on metabolism (3, 17); on the influence of the degree of fatness on the utilization of feed (5); on the relative utilization of feed energy for maintenance, body increase, and milk production (13, 18); on the influence of environmental temperature on the heat production (9); on the energy metabolism in relation to the plane of nutrition (10, 11); on the analysis of the curve of heat production (15, 16); and on the balance of nutrients, that is, the supplementing effects of feeding stuffs in combination, as affecting the utilization of feed energy (7, 12).

Among the various feeding stuffs investigated at this institute corn (maize) meal has received relatively much attention, and the methods employed in measuring its net energy embrace the various procedures and problems involved in this estimation as applied to feeding stuffs in general, but especially to feeds of concentrated character.

The general point of view expressed by these methods and the earlier of the detailed procedures originated with Armsby. During the decade since his death these procedures have been continuously studied and frequently modified, with the hope of improvement, since no method for determining the net energy of individual feeding stuffs had, or has, been found that can be relied upon for concordant and defensible results.

The first attempt to determine the net energy value of corn meal was made by Armsby and Fries in the second of their respiration-calorimetric studies, in 1902 (1). The results obtained with a steer on a basal ration of red-clover hay were compared with the results obtained when to this basal ration corn meal in different quantities

<sup>1</sup> Received for publication Oct. 31, 1932; issued May, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited p. 769.

was added, in two experimental periods. This procedure is usually referred to in recent discussion as the "feed-and-plane-difference method," since there was a difference not only of kind but also of quantity in the rations compared. The values obtained for corn meal in this experiment were widely divergent, and only one of them could be considered as plausible.

Subsequently, Armsby and Fries repeated the determination of the net energy value of corn meal in two series of experiments. In one of these (6) corn meal was combined with red-clover hay, and in the other (4) mixed hay was used as the roughage. In both of these series of experiments, however, the net energy was determined on the mixed ration by feeding it at two planes of intake; the net energy of the roughage was determined by feeding alone, at two planes of intake; and the net energy of the corn was computed by a difference process depending on the assumption that the value of the roughage in the mixed ration was the same as when fed alone. This procedure has been shown to have certain advantages (19) over the earlier one, notably in that there is likely to be less exaggeration of experimental errors. It disregards, however, the influence of the plane of nutrition on the utilization of feed energy, which has been shown to be an important factor in this relation (10, 11).

The values obtained for corn meal in these experiments were diverse and thus could not be considered reliable.

Since the adoption of the directly observed fasting katabolism of cattle as a measure of the maintenance requirement of energy, and as a base value in energy metabolism, the writers have endeavored in several series of experiments (10, 11) to determine the net energy value of corn meal both for maintenance and for body increase, employing procedures that give full recognition to the factor of plane of nutrition. The essential feature of one of these procedures, the "heat-increment-proportional method," is the assumption that the heat-increment value of an individual feeding stuff for maintenance is to its heat-increment value for body increase as the heat-increment value of a mixed ration, of which it is a component, for maintenance, is to the heat-increment value of the same ration for body increase.

Another procedure, the "net-proportional method," assumes that the net energy value of a single feeding stuff for maintenance is to its net energy value for body increase as the net energy of a mixed ration, of which it is a component, for maintenance, is to the net energy of the mixed ration for body increase.

Neither of the above-mentioned methods yielded consistent net energy values of single feeding stuffs for body increase. In fact, the results obtained strongly suggested associative effects of the feeding stuffs fed in combination, on the utilization of their energy, in addition to the influence of differences in the plane of nutrition of the rations compared.

The first direct demonstration of such associative effects of feeding stuffs was obtained by the writers in subsequent experiments (12) in which corn meal was fed exclusively and in combination with alfalfa hay, at a maintenance level. The heat increment of the corn meal when fed alone was found to be considerably greater (about three times) than that computed for it from results obtained when the corn meal was fed combined with the hay, while the metab-



olizable energy value of the corn meal was practically the same when it was fed alone as when fed with hay.

In the present investigation corn meal was added, in different experimental periods, to three basal maintenance rations differing radically in composition, thus providing for the further study of the early, simple, and direct feed-and-plane-difference method of Armsby for determining net energy values of individual feeding stuffs, and also for an investigation of the fundamental problem of associative or supplementing effects of feeds in combination.

The mixed rations resulting from the combination of corn meal with the basal maintenance rations used are unquestionably better balanced, nutritively, than is corn meal alone, and the effects of the combination are logically reciprocal; but inasmuch as there is no scientific method for apportioning these effects, the differentiation of the values of the basal ration and the supplementary corn meal is possible only if it be assumed that one of these two ration components is the active agent and the other is acted upon.

In the light of general knowledge the three basal rations employed were considered to be of poor, of low-medium, and of excellent nutritive value. The net energy value given to the corn meal by the supplementing effect of the combination of corn meal and these basal rations would obviously be in accord with the effectiveness of the reciprocal supplementation, and not necessarily in accord with the nutritive balance of the basal rations.

In other words, an unbalanced basal ration fed with corn meal might give to the corn meal a higher net energy value, if the basal ration were unbalanced in a way to balance the corn meal, than would a perfectly balanced basal ration fed with corn meal.

Theoretically, at least, any "effective" deficiency of any essential nutrient must eventually affect the utilization of the energy of the food, and the question whether a specific nutritive deficiency is effective at a particular time obviously depends on (1) the nature of the deficiency, (2) the extent of the requirement of the animal for the deficient nutrient, (3) the extent and availability of the body reserve of the nutrient which is deficient in the ration, and (4) the length of the period of experimentation.

Thus, since corn meal is characterized by a high metabolizable energy value, but by marked nutritive deficiencies, as previously noted, a question of importance is whether, in the course of a 28-day feeding period, these deficiencies affect the utilization of the energy of the corn meal in an unfavorable manner, or whether the animal that has eaten the corn meal is either partly or wholly protected from these deficiencies, by drafts upon the nutritive reserves of its body, so that the deficiencies are without apparent or immediate effect on the net energy value of the corn meal.

In this relation it would be exceedingly important to know how quickly and to what extent specific nutritive deficiencies affect the utilization of the energy of feeding stuffs. For instance, a deficiency of protein in a ration might conceivably affect the utilization of its energy within one day, a deficiency of vitamin B within a week, a deficiency of vitamin A within a month, a deficiency of phosphorus within 6 months, and a deficiency of vitamin D not at all, while the food consumption remains unaffected.

Also, an inorganic compound, calcium phosphate, for instance, or a vitamin in an experiment of adequate length might "earn" a net energy value if added to a ration which is deficient in the substance but would have no energy value in other relations.

Furthermore, the senior writer learned many years ago that in feeding for body increase the smaller the proportion in which a protein supplement to corn is employed the greater will be its apparent value, per unit of itself.

From the foregoing it will be understood that in the authors' opinion much remains to be learned as to the effects of specific kinds and degrees of nutritive deficiency upon net energy values, and as to the time element in such relations. Also, conceding that different net energy values will be found (as affected by drafts upon nutritive reserves of the animal body) in experimental periods of different length, there remains the question, What would be the most nearly true or characteristic value, or the one of greatest significance, as applying to particular conditions of feeding practice?

#### PLAN OF EXPERIMENTATION

A duplicate series of seven energy-balance experiments was conducted during the fall, winter, and spring of 1931-32, with two 2-year-old steers, as follows: (1) During fast; (2) at the maintenance level, with three rations of different character; and (3) at a production level, the rations being the same as the three fed at the maintenance level plus corn meal in quantities equivalent to one-half of the estimated maintenance requirement of energy.

The schedule of experimentation is given in Table 1, together with the daily rations, and the live weights of the experimental subjects.

TABLE 1.—Schedule of experimentation, daily rations, and live weights of animals in experiment No. 261

Period No.	Steer	Preliminary experimental feeding period	Periods of excreta collection	Calorimeter periods	Plane of nutrition	Rations fed daily							Average live weight of animals
						Oat straw	Timothy hay	Alfalfa hay	Corn meal	Linseed meal	Wheat bran	Molasses	
		Days	Days	Days		Kg	Kg	Kg	Kg	Kg	Kg	Kg	Kg
1	A	Days	Days	Days	Fasting								385.3
2	B				do								318.8
3	A	4	12	3	Maintenance	2.500			1.800			0.180	393.5
4	B	8	21	3	do				1.640			.160	323.6
5	A	4	10	3	1½ maintenance	2.500			3.268			.180	409.1
6	B	4	10	3	do	2.290			2.982			.160	335.5
7	A	9	21	3	Maintenance		2.976		1.502				401.0
8	B	10	21	3	do		2.590		1.308				325.8
9	A	14	21	3	1½ maintenance		2.976		3.004				425.1
10	B	14	21	3	do		2.590		2.616				343.2
11	A	8	21	3	Maintenance			3.500		0.910	0.910		432.2
12	B	9	21	3	do			3.024		.786	.786		351.7
13	A	9	21	3	1½ maintenance			3.500	1.538	.910	.910		453.1
14	B	14	21	3	do			3.024	1.348	.786	.786		372.7

The three basal maintenance rations differed widely as to protein, mineral, and, presumably, as to vitamin contents. The first ration

consisted of oat straw and corn meal (1.4:1), supplemented with a small quantity of molasses to increase its palatability; the second consisted of timothy hay and corn meal (2:1); while the third was made up of alfalfa hay, linseed meal, and wheat bran in a proportion of 3.8:1:1.

Inasmuch as the maintenance rations supplied nutriment approximately as required to sustain energy equilibrium, the results obtained for the added corn meal, in the production periods, were comparable in that the plane of nutrition was essentially the same in all three cases.

The fasting experiments with the two animals provided a basis for determining the energy cost of maintenance, and of standing as compared with lying. This information was required in computing the heat production of the animals, standing or lying, in all experimental periods to a standard day, and to the basis of a uniform live weight.

In the fasting periods the heat production and the gaseous exchange were measured continuously in the respiration calorimeter for four consecutive days, and the attainment of the status of true fast was indicated by the respiratory quotients, which were determined in intermittent samples of the air current taken every three hours.

The experimental periods during which the animals received feed consisted of a preliminary feeding period of 9 to 14 days, a digestion period ordinarily 21 days in length, and a calorimeter period to 3 days—usually the last 3 of the digestion period.

Each calorimeter period was preceded by a preliminary period of 14 hours, during which the experimental ensemble was established in adiabatic balance. The animal was inside the calorimeter during this time.

In three cases (periods 3, 5, and 6) the digestion periods as well as the preliminary periods were of necessity considerably shortened, as indicated in Table 1, because of unsatisfactory behavior of the experimental subjects.

During the digestion periods quantitative collections were made of feces and urine, while during the calorimeter periods, in addition to the collection of the visible excreta, the gaseous products and the heat production of the animals were measured.

In all the feeding periods the heat production was measured directly as usual, and checked by indirect determination from the carbon, nitrogen, and energy balances. Final comparisons and computations, in the utilization of the results, were based on the average of the direct and the indirect measurements.

#### EXPERIMENTAL SUBJECTS

The steers used as subjects in these experiments were designated A and B. Steer A was a crossbred Aberdeen-Angus-Shorthorn, born January 25, 1930, and steer B was a purebred Shorthorn, born April 16, 1930. Their average live weights in the first two experimental feeding periods (Nos. 3 and 4) were 393.5 kg and 323.6 kg, respectively.

#### EXPERIMENTAL DATA

The chemical composition and digestibility of the rations, and of three of the individual feeding stuffs, corn meal, oat straw, and timothy hay, are given in Table 2.

TABLE 2.—Chemical composition and digestibility of rations, and of corn meal, oat straw, and timothy hay

Period No.	Steer	Ration No. or feeding stuff	Chemical composition (dry basis)					Digestion coefficients				
			Crude protein	Carbohydrate		Ether extract	Energy per gram	Crude protein	Carbohydrate		Ether extract	Energy
				Nitrogen-free extract	Crude fiber				Nitrogen-free extract	Crude fiber		
			Per cent	Per cent	Per cent	Per cent	Calories	Per cent	Per cent	Per cent	Per cent	Per cent
3	A	3	7.01	56.48	24.35	3.23	4.375	46.7	72.8	54.6	61.8	62.2
4	B	4	7.00	56.43	24.41	3.23	4.373	44.6	70.7	50.3	58.3	59.7
5	A	5	7.34	62.24	19.41	3.82	4.461	52.9	75.6	51.4	72.3	66.3
6	B	6	7.34	62.22	19.46	3.82	4.459	41.3	71.9	50.7	63.7	63.6
7	A	7	7.14	61.07	24.10	3.19	4.497	54.7	71.9	46.9	66.5	62.0
8	B	8	7.00	61.02	24.27	3.14	4.490	42.9	69.8	42.9	63.1	58.9
9	A	9	7.47	65.69	19.49	3.62	4.511	56.8	77.6	45.4	70.1	66.0
10	B	10	7.51	65.46	19.73	3.58	4.503	43.4	75.7	41.3	63.4	62.2
11	A	11	22.13	43.06	23.33	3.24	4.536	79.7	77.0	41.9	58.2	65.1
12	B	12	22.10	43.03	23.31	3.23	4.532	77.9	76.1	33.9	57.4	62.1
13	A	13	19.54	51.13	18.86	3.67	4.539	76.9	82.5	34.0	65.8	67.9
14	B	14	19.47	51.23	18.78	3.67	4.536	76.8	81.9	26.5	64.4	66.2
5	A	Corn meal	9.59	81.81	2.03	5.11	4.538	70.6	82.3	(-66.9)	92.3	79.5
9	A	do.	9.59	81.81	2.03	5.11	4.538	62.7	91.5	(19.5)	77.4	78.8
13	A	do.	9.94	81.13	2.20	5.28	4.551	54.0	93.2	(-241.8)	83.0	78.3
6	B	do.	9.59	81.81	2.03	5.11	4.538	(32.1)	(74.8)	(62.5)	(73.9)	(76.0)
10	B	do.	9.94	81.13	2.20	5.28	4.551	44.7	90.1	(19.0)	64.1	72.3
14	B	do.	9.94	81.13	2.20	5.28	4.551	68.1	92.9	(-216.4)	79.9	80.6
3	A	Oat straw	5.13	43.45	41.91	2.56	4.483	(12.3)	(60.1)	(58.5)	(23.3)	(48.3)
5	A	do.	5.14	43.00	42.50	2.53	4.490	(12.3)	(60.1)	(58.5)	(23.3)	(48.3)
4	B	do.	5.13	43.45	41.91	2.56	4.483	(62.5)	(65.2)	(50.0)	(38.6)	(46.4)
6	B	do.	5.14	43.00	42.50	2.53	4.490	(62.5)	(65.2)	(50.0)	(38.6)	(46.4)
7	A	Timothy hay	6.06	52.05	34.68	2.33	4.528	(48.0)	(57.2)	(47.2)	(55.1)	(54.1)
9	A	do.	5.56	51.30	36.09	2.27	4.537	(48.0)	(57.2)	(47.2)	(55.1)	(54.1)
8	B	do.	5.86	52.00	35.07	2.25	4.527	(41.4)	(64.5)	(43.1)	(61.8)	(52.6)
10	B	do.	5.31	51.50	36.55	2.02	4.514	(41.4)	(64.5)	(43.1)	(61.8)	(52.6)

If the rations are designated by the experimental period numbers, rations 3, 4, 5, and 6, composed of corn meal, oat straw and molasses, may be characterized, in the light of general information, as unpalatable and nutritively deficient, especially because oat straw is a very poor roughage, although a great deal of it is eaten by cattle.

Rations 7, 8, 9, and 10, composed of corn meal and timothy hay, may be regarded as of low-medium quality. These rations, like those in the preceding group, are deficient in variety of components, and timothy hay is a second-rate roughage for cattle.

Rations 11, 12, 13, and 14 were of high grade. The roughage, alfalfa hay, was of the best quality, and the grain components were palatable, varied, and nutritious. The rations were rich in protein, calcium, and phosphorus and are not known to have been lacking in any essential nutrient.

In these three groups of rations, therefore, corn meal was fed in decidedly poor, in low-medium, and in excellent combinations.

The protein intake differed much from period to period but was, as intended, at all times safely above the maintenance requirement, as shown by the invariably positive nitrogen balances. (Table 4.) The nitrogen intake, in grams, per kilogram of live weight of steers A and B in the several experimental periods was as follows: Periods 3 and 4, 0.117 and 0.129; 5 and 6, 0.151 and 0.168; 7 and 8, 0.114 and

0.119; 9 and 10, 0.147 and 0.160; 11 and 12, 0.395 and 0.420; and 13 and 14, 0.424 and 0.447.

The ratios of carbon to nitrogen in the basal rations containing oat straw, timothy hay, and alfalfa hay, respectively, were 39 to 1, 41 to 1, and 13 to 1. In the supplemented rations (with corn meal) these ratios were 38 to 1, 39 to 1, and 15 to 1, respectively.

The energy of the digestible nutrients of rations 5 and 6 was greater than that of rations 3 and 4, on account of the added corn meal which the former contained. A similar difference prevailed, for the same reason, between rations 9 and 10 as compared with 7 and 8, and between rations 13 and 14 as compared with 11 and 12.

In each of the six cases in which the same ration was fed to the two subjects steer A digested an appreciably greater part of the energy-producing nutriment of the ration than did steer B, the differences, as shown in Table 2, being 2.5, 2.7, 3.1, 3.8, 3.0, and 1.7 per cent, respectively. Also, without exception, steer A digested all other kinds of nutriment in each ration more efficiently than did steer B.

This comparison, therefore, shows that individuality is a significant factor affecting digestible nutrient and therefore net energy values.

The digestibility of the corn meal by the two steers, as affected by the feeds with which it was fed, is given in Table 2. Inasmuch as the digestibility of the corn meal was of necessity computed by a difference or subtraction procedure, which served to relate and to express all errors of theory and of work as though affecting only the values of the comparatively small quantities of corn meal; and, further, inasmuch as the computation of the coefficients for corn meal depended on the assumption (which is not exactly true) that the digestibility of the corn meal and of the basal part of the ration in each case was the same when fed separately as when fed together, marked inconsistencies appear in these computed digestion coefficients. These inconsistencies are only the beginning of the troubles encountered in efforts to determine net energy values of individual feeding stuffs.

The first three of these values for corn meal were obtained, steer A being the subject, in periods 5, 9, and 13, while steer B was the subject in periods 6, 10, and 14. Manifestly unrepresentative data, or data which for any reason are considered questionable, are inclosed in parentheses. The unreliability of the data in period 6 was due to the fact that the animal did not take the feed satisfactorily, the collection period was short, and the correspondence between the ration and the feces was obviously imperfect.

In considering these six digestion coefficients for corn meal a comparison should be made of the performance of the two steers in periods 5 and 6, 9 and 10, and 13 and 14. In view of the foregoing observations, however, these digestion coefficients of corn meal can not be closely interpreted.

In other words, digestion coefficients of individual feeding stuffs, computed by difference, must be considered as conventional—especially because the digestibility of foodstuffs given individually may not be the same as when fed in combination and because there is no scientific method for apportioning the digestibility of a mixed ration among its component feeding stuffs.

The individual digestion coefficients of oat straw and timothy hay were also computed, by assuming that the values for corn meal,

representing the difference between two rations, apply also to the corn meal within these rations. The second difference process involved in this computation serves still further to magnify the errors incident to the first, and the values thus derived for the roughages are obviously without definite significance. The process of computation used is superficially logical, but, as explained, the effects of the errors of work and of fundamental assumption render the results quite imperfectly representative.

TABLE 3.—Carbon dioxide, water vapor, methane, and heat production per day

Period No.	Steer	Calorimeter day	CO <sub>2</sub>	H <sub>2</sub> O	CH <sub>4</sub>	Heat by radiation and conduction <sup>a</sup>	Latent heat of H <sub>2</sub> O vapor	Total heat				
								Directly observed	Directly observed, corrected for body gain	Indirectly computed	Average, computed and observed	Corrected to standard day
			Grams	Grams	Grams	Calories	Calories	Calories	Calories	Calories	Calories	Calories
3	A	First.....	3,697.4	---	---	109.2	5,210	9,127	---	---	---	---
		Second....	3,816.6	---	---	113.9	5,510	9,427	---	---	---	---
		Third.....	3,816.9	---	---	114.5	5,704	9,621	---	---	---	---
		Average...	3,777.0	6,684	---	112.5	5,475	9,392	9,275	9,176	9,226	9,298
4	B	First.....	3,411.6	---	---	106.0	5,234	8,414	---	---	---	---
		Second....	3,383.4	---	---	103.1	5,324	8,504	---	---	---	---
		Third.....	3,414.2	---	---	99.6	5,425	8,605	---	---	---	---
		Average...	3,403.1	5,426	---	102.9	5,328	8,508	8,509	8,285	8,397	8,632
5	A	First.....	4,369.2	---	---	139.8	7,969	10,414	---	---	---	---
		Second....	4,472.8	---	---	140.9	7,936	10,381	---	---	---	---
		Third.....	4,410.5	---	---	143.2	7,903	10,348	---	---	---	---
		Average...	4,417.5	4,165	---	141.3	7,936	10,381	10,333	10,138	10,236	10,538
6	B	First.....	3,924.4	---	---	122.5	5,807	9,182	---	---	---	---
		Second....	3,992.4	---	---	123.8	6,094	9,469	---	---	---	---
		Third.....	4,091.4	---	---	122.5	6,298	9,673	---	---	---	---
		Average...	4,002.7	5,750	---	122.9	6,066	9,441	9,481	9,218	9,481	9,645
7	A	First.....	3,708.3	---	---	111.1	6,973	8,993	---	---	---	---
		Second....	3,642.7	---	---	110.9	6,915	8,935	---	---	---	---
		Third.....	3,632.4	---	---	109.2	6,718	8,732	---	---	---	---
		Average...	3,661.1	3,440	---	110.4	6,869	8,837	8,862	8,768	8,815	9,015
8	B	First.....	3,181.5	---	---	93.5	5,688	7,745	---	---	---	---
		Second....	3,107.5	---	---	89.9	5,478	7,535	---	---	---	---
		Third.....	3,122.9	---	---	91.3	5,438	7,495	---	---	---	---
		Average...	3,137.3	3,504	---	91.6	5,355	7,592	7,618	7,685	7,652	7,985
9	A	First.....	4,553.9	---	---	144.9	6,862	10,604	---	---	---	---
		Second....	4,596.5	---	---	143.8	7,053	10,795	---	---	---	---
		Third.....	4,630.5	---	---	139.3	7,163	10,905	---	---	---	---
		Average...	4,593.6	6,374	---	142.7	7,026	10,768	10,698	10,669	10,684	10,885
10	B	First.....	3,925.2	---	---	111.2	6,321	9,417	---	---	---	---
		Second....	4,053.6	---	---	113.5	6,593	9,689	---	---	---	---
		Third.....	3,957.1	---	---	109.7	6,543	9,639	---	---	---	---
		Average...	3,975.6	5,275	---	111.5	6,486	9,582	9,577	9,403	9,490	9,637
11	A	First.....	4,256.4	---	---	118.0	7,137	10,754	---	---	---	---
		Second....	4,276.2	---	---	120.6	6,940	10,557	---	---	---	---
		Third.....	4,188.0	---	---	117.0	6,802	10,419	---	---	---	---
		Average...	4,240.2	6,162	---	118.5	6,960	10,577	10,504	10,371	10,438	10,598
12	B	First.....	3,742.9	---	---	98.2	6,632	9,604	---	---	---	---
		Second....	3,684.8	---	---	96.9	6,142	9,114	---	---	---	---
		Third.....	3,752.0	---	---	95.4	6,585	9,557	---	---	---	---
		Average...	3,726.6	5,063	---	96.8	6,453	9,425	9,488	9,375	9,432	9,511
13	A											
14	B	First.....	4,483.1	---	---	133.8	6,667	10,586	---	---	---	---
		Second....	4,606.0	---	---	136.2	7,276	11,195	---	---	---	---
		Third.....	4,543.0	---	---	139.4	6,860	10,779	---	---	---	---
		Average...	4,544.0	6,688	---	136.5	6,934	10,853	10,858	10,798	10,828	10,944

<sup>a</sup> Heat measured by water current diminished by heat liberated on condensation of water on absorbers.

<sup>b</sup> Based on one 12-hour subperiod.

<sup>c</sup> Observed heat only.

The data for carbon dioxide, water vapor, methane, and heat produced are given in Table 3, the heat production being computed,

finally, to the basis of the standard day of 12 hours standing and 12 hours lying, and to the basis of a standard live weight.

The data for gaseous outgo and heat production of steer A in period 13 are omitted, because they were vitiated by unfavorable experimental conditions. The difficulty apparently began with the steer's shedding hair into his urine funnel, which clogged the flow of urine and led to its overflowing onto the floor on which the steer stood or lay. For this reason the steer was uncomfortable and changed position (as to standing and lying) 62 per cent more times than did steer B on the same ration. He developed the habit of standing out of position with his forefeet up on the lid of the feed box. This disarranged the feces apron, and feces were spilled onto the floor.

The air in the calorimeter chamber became so strongly ammoniacal that the drip water from the copper heat absorbers was blue. The attendant had to enter the chamber seven times during the 3-day period to rearrange the harness and the stall appurtenances.

In these experiments the daily elimination of carbon dioxide, methane, and heat varied but little during the three days of each calorimetric measurement.

The rate of ventilation, however, was less than that which ordinarily prevailed in the experiments of previous years, and considerable quantities of water condensed on the absorber pipes. The water remaining in the air current was fairly constant in quantity; but that which was condensed on the absorbers, and consequently the total eliminated, appeared to vary much from day to day, since the flow of condensed water from this apparatus is not accurately quantitative for 24-hour periods. The quantities of this condensed water, as observed on the separate days of a 3-day calorimetric measurement, therefore, were averaged, as were also the accurately measured quantities of moisture carried from the calorimeter in the air stream, these two portions being added to give the quantity of the total outgo of water vapor.

TABLE 4.—*Intake and balance of matter and energy*

Period No.	Steer	Daily intake of—						Daily balances of—					
		Dry matter	Water	Nitrogen	Carbon	Gross energy	Metabolizable energy	Water	Nitrogen	Carbon of protein	Carbon of fat	Total carbon	Energy
		<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>	<i>Calories</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Calories</i>
3.....	A	3,954	10,256	46.0	1,811	17,429	8,767	-5,792	8.4	26	-51	-25	-409
4.....	B	3,610	14,300	41.9	1,654	15,917	7,646	133	6.3	20	-65	-45	-639
5.....	A	5,103	11,208	61.6	2,366	22,899	12,659	-2,503	16.7	53	167	220	2,521
6.....	B	4,661	16,601	56.2	2,161	20,918	11,155	1,749	13.0	41	128	169	1,937
7.....	A	3,970	7,948	45.6	1,863	17,986	8,980	-1,230	5.6	18	5	23	212
8.....	B	3,428	11,487	38.7	1,605	15,528	7,410	1,314	4.1	13	-31	-18	-275
9.....	A	5,206	10,274	62.6	2,436	23,620	12,891	-3,504	19.0	60	138	198	2,222
10.....	B	4,530	16,133	54.8	2,122	20,532	10,656	-321	9.7	31	80	111	1,253
11.....	A	4,801	16,856	170.9	2,222	21,914	10,978	-3,601	16.2	51	14	65	607
12.....	B	4,148	22,158	147.7	1,920	18,934	9,025	3,102	12.0	38	-54	-16	-350
13.....	A	6,116	20,795	192.1	2,833	27,899	14,850	24.4	24.4				
14.....	B	5,315	23,706	166.6	2,462	24,245	12,558	135	22.6	71	93	164	1,760

The intake and the balance of matter and energy are given in Table 4. It is of interest to note that the nitrogen balances for both animals were all positive and were of significant magnitude even in

the energy-maintenance periods on the low-protein rations consisting of oat straw, corn meal, and molasses (periods 3 and 4), and on those made up of timothy hay and corn meal (periods 7 and 8). The daily balances of energy in the maintenance periods, however, were comparatively small and show that the estimates of energy requirement employed in computation of the rations were satisfactory.

The quantities of nitrogen excreted in the urine during the first eight periods, when the animals were on the low-protein rations, are much less than as usually observed, because of the low protein intake and the sparing effect of carbohydrates on protein metabolism. When the animals were on the oat straw-corn meal rations (periods 3 to 6, inclusive) the daily urinary nitrogen was 13.8 g, 13.0 g, 16.5 g, and 10.9 g, respectively, which is equivalent to an average of 3.8 g per 100 kg of body weight for steer A, and to an average of 3.6 g for steer B; but when they received the timothy hay-corn meal rations (periods 7 to 10, inclusive) the daily urinary nitrogen was 19.3, 12.5, 16.6, and 14.1 g, respectively, which is equivalent to an average of 4.3 g per 100 kg of body weight for steer A, and an average of 4.0 g for steer B. In previous experiments (10) the average daily nitrogen excretion of four steers which received no feed was found to be 8.1 g, 9.0 g, 11.3 g, and 9.8 g, respectively, per 100 kg of body weight.

It is a thoroughly established fact that the urinary-nitrogen excretion during fast does not represent the minimum protein requirement of the animal, since during fast some protein is katabolized for energy production. Many workers have observed that on protein-free rations, or on rations very poor in protein, the protein katabolism is reduced to a level considerably lower than that which prevails during fast.

The data for urinary nitrogen during fast in this series of experiments are not reported, but during periods 3, 4, and 8 the nitrogen requirement, as measured by the digestible nitrogen minus the positive nitrogen balance (the remainder being equal to the urinary nitrogen) was 13.8, 13.0, and 12.5 g, respectively, which is equal to 3.5, 3.1, and 3.8 g per 100 kg of live weight, or to an average of 21.7 g of crude protein. This is less than half of the protein allowed for maintenance that is indicated by prevailing feeding standards for cattle.

The quantities of nitrogen excreted in the urine of the animals on the high-protein rations (periods 11 to 14, inclusive) are about 8 to 10 times as great as those when they were on the low-protein rations. The carbon-nitrogen ratios in the urine in periods 11 to 14 (high-protein rations) are 1.2, 1.2, 1.3, and 1.3, respectively, while the carbon-nitrogen ratios in the urine in periods 3 to 10 (low-protein rations) are 3.6, 3.2, 3.2, 3.7, 3.3, 3.8, 3.9, and 4.0, respectively. These observations indicate that under a given set of conditions the carbon-nitrogen ratio in the urine is fairly constant, but that there is no fixed relation between the carbon content of the urine and the quantities of protein katabolized, and that some of the carbon in the urine represents nonnitrogenous substance.

In computing the intake and the balance of matter and energy the energy of the urine and of the protein gained or lost was corrected, as usual, for incomplete oxidation of protein, and metabolizable energy was determined as the gross energy of the feed minus the energy of the feces, of the methane, and of the urine, corrected as above stated.



The data entering into the determination of the balances of matter and energy are regarded as satisfactory, except as unfavorable conditions vitiated the significance of the heat production and related data in period 13, and except for the data representing the feces in period 6. Conditions in the latter period were unsatisfactory in that it was impossible, because of refusal of feed by the subject, to have as long an experimental period as usual. The basis of separation of the intervals of preliminary feeding from the period of excreta collection was uncertain, since the quantity of feces eliminated varied greatly from day to day. In order, therefore, to arrive at the most plausible value for metabolizable energy for use in computing the net energy the preliminary feeding interval was made eight days, and the period of excreta collection six days, this separation being determined by the fact that it yielded a metabolizable energy value of corn meal in agreement with the values that were satisfactorily determined in the other experimental periods.

Seven values for heat production are given in Table 3. The heat by radiation and conduction is the heat as measured in the stream of water which carries the heat from the calorimeter chamber, diminished by the quantity of heat liberated on condensation of water on the absorbers.

The latent heat of water vapor was computed from the average of the daily measurements of water vapor in the air current, plus the average daily quantity liberated by condensation of water on the absorbers.

The total heat directly observed is the sum of these values for heat of radiation and conduction and latent heat of water vapor. This value for total heat directly observed was then subjected to a minor correction for heat stored in or lost from the body in the gain or loss of body substance.

The total heat indirectly computed was derived from the balances of carbon, nitrogen, and energy and represents the algebraic difference between the metabolizable energy of the feed and the energy of body increase.

A crucial test of the validity of the heat measurements is afforded by a comparison of the directly measured and the indirectly computed data, since these values are derived by entirely different methods and technic. In these experiments this agreement is remarkably good, which shows that the heat measurements must have been essentially correct, and this fact should be borne in mind in the interpretation of the final results. The agreement of the directly and the indirectly determined heat production, expressed in percentage of the direct heat, (including the values for period 13, which were finally discarded), was on an average 98.7 per cent, the extremes being 97.3 and 100.9 per cent. Since there was no significant difference between these values they were averaged, and subsequent computations are based on this average figure.

The last column of Table 3 gives this average heat measurement corrected, for comparative purposes, in accordance with the established procedure of this institute, to a standard day of 12 hours standing and 12 hours lying, in order to eliminate the effect on heat production of differences of time spent by the animals in these positions.

This correction for standing and lying was based on factors experimentally determined during the fasting periods (Nos. 1 and 2),

representing the relative rates of  $\text{CO}_2$  production during standing and lying, in accordance with a procedure described in a previous paper (17). For steer A the difference in heat produced per hour of standing, as compared with that produced while lying, was 13.1 Calories, while for steer B this difference was 19.8 Calories per hour per 100 kg body weight.

The figures for heat production in periods 1 and 2 represent, respectively, the third day of fast for steer A, and the fourth day of fast for steer B. These days were selected because they were the first days during which the nonprotein respiratory quotients represented the katabolism of fat alone, thus indicating a true fasting status.

A final correction of the heat production, for differences in live weight, is indicated in the fifth column of figures in Table 5, this correction representing the differences in fasting katabolism (maintenance requirement) related to the differences in the two-thirds power of the live weights in the several periods. The effect of these corrections, therefore, is to reduce the maintenance requirements of the animals, in the various periods, to the basis of a uniform live weight.

In consideration of the contents of the alimentary tract, the values actually used in this computation to represent the body weight of the fasting animals were not those observed during fast but were the average weights obtained during the periods of maintenance immediately following fast (period 3, for steer A, and period 4, for steer B), diminished by the amount of the loss in body tissue, which was computed to be 2 kg per day (10). It is believed that this procedure renders the live weights more nearly comparable in regard to the "fill" than would the use of the actual weight of the fasted animals.

In applying the corrections for standing, and for differences in live weight, the average of the observed and of the computed heat production was used in all feeding periods, except period 6. In this period the observed heat production was considered more reliable—for reasons already given.

#### METABOLIZABLE ENERGY, HEAT INCREMENT, AND NET ENERGY VALUES OF CORN MEAL FOR BODY INCREASE

In Table 5 are given the metabolizable energy, heat-increment, and net energy values of corn meal for body increase, as influenced by the other components of the rations in which the corn meal was fed.

These values were derived by a comparison of data obtained from the basal maintenance rations with parallel observations from the production rations, which were the same as the former plus corn meal.

Three such comparisons were possible for each animal—periods 3 and 5, 7 and 9, 11 and 13, for steer A, and periods 4 and 6, 8 and 10, 12 and 14, for steer B. In each case the differences in the metabolizable energy, in the heat production, and in the gains of energy have been ascribed to the corn meal in the production rations and have been expressed in relation to kilograms of dry matter. Actually there were slight differences in the dry matter of the basal components of the rations compared, on account of slight differences in moisture content, but these differences were considered negligible in relation to this computation.

TABLE 5.—Metabolizable energy, heat increment, and net energy values of corn meal

Period No.	Steer	Plane of nutrition, and kind of roughage in ration	Dry matter of daily ration	Dry matter of added corn <sup>a</sup>	Metabolizable energy		Total heat computed to standard day and live weight	Heat increase per kilogram of dry matter of added corn	Energy balance	Net energy per kilogram of dry matter of corn added to basal ration
					Total	Per kilogram of dry matter of corn added to basal ration				
3	A	Maintenance, oat straw	Grams 3,954	Grams	Calories 8,767	Calories	Calories 9,233	Calories -466	Calories	Calories
5	A	1.5 maintenance, oat straw	5,103	1,149	12,659	3,387	10,305	933	2,354	2,454
4	B	Maintenance, oat straw	3,610		7,646		8,530		-884	
6	B	1.5 maintenance, oat straw	4,661	1,051	11,155	3,339	9,393	821	1,762	2,518
7	A	Maintenance, timothy hay	3,970		8,980		8,869		111	
9	A	1.5 maintenance, timothy hay	5,206	1,236	12,891	3,164	10,483	1,306	2,408	1,858
8	B	Maintenance, timothy hay	3,428		7,410		7,854		-444	
10	B	1.5 maintenance, timothy hay	4,530	1,102	10,656	2,946	9,288	1,301	1,368	1,645
11	A	Maintenance, alfalfa hay	4,801		10,978		10,122		856	
13	A	1.5 maintenance, alfalfa hay	6,116	1,315	14,850	2,944				
12	B	Maintenance, alfalfa hay	4,148		9,025		9,056		-31	
14	B	1.5 maintenance, alfalfa hay	5,315	1,167	12,558	3,027	10,233	1,009	2,325	2,018

<sup>a</sup> This value, determined as the difference between 2 rations, is very slightly in error as representing the corn meal added to the basal ration owing to the fact that while the basal ration and the basal components of the supplemented ration were identical, on the fresh basis, they differed slightly in moisture content in the 2 rations (periods) compared.

The net energy value of the corn meal is the difference between the metabolizable energy value and the heat increment value; or, it may be said to represent the difference in the gain of energy between the two periods compared, related to the difference in feed (corn meal).

This method of computation of the net energy value of a single feeding stuff, for body increase, was proposed by Armsby and was employed by him in many of his experiments. It is the simplest and most direct of the methods available for this purpose, and in this series of experiments is the only method usable. It has, however, some undesirable features which must be recognized. These have been discussed at some length by one of the writers elsewhere (19). The important fact to bear in mind in this connection is that in case the difference in feed consumed in the periods compared is small, this method of computation may cause extensive exaggeration of experimental errors.

The metabolizable energy values of the corn meal exhibited appreciable variation, the highest value being obtained when the corn meal was fed in combination with the basal ration containing oat straw.

The most striking results of these experiments are the very different heat increment values obtained for corn meal in the different comparisons made. Thus, for steer B the heat increment values

for corn meal in the three rations containing oat straw, timothy hay, and alfalfa hay were 821, 1,301, and 1,009 Calories., respectively, while for steer A the corresponding values for the rations containing oat straw and timothy hay were 933 and 1,306 Calories, respectively.

As a result of the variations observed in the metabolizable energy values, and in the heat increment values, the net energy values of the corn meal for body increase also vary greatly. When the corn meal was combined with the basal ration of oat straw and corn meal the net energy value per kilogram of dry matter of the added meal was found to be 2,454 Calories for steer A, and 2,518 Calories for steer B. In the timothy-hay corn-meal combinations the net energy value of the meal was found to be 1,858 Calories for steer A, and 1,645 Calories for steer B. When the corn meal was added to the high-protein basal ration containing alfalfa hay the net energy value of the meal was 2,018 Calories for steer B, and for reasons already explained there is no value given for steer A. These results, therefore, show maximum variation in the apparent net energy value of corn meal for body increase, at comparable planes of nutrition, with a single experimental subject, between 1,645 and 2,518 Calories.

These differences in metabolizable energy, heat increment, and net energy values of corn meal for body increase may be regarded mainly as results of mutual nutritive supplementation of the ration components, but also in part as results of the individuality of the experimental subjects and of arithmetical exaggeration of experimental error, the principles of which have been discussed at length (11).

The plan of experimentation was not such as to reveal the degrees of nutritive supplementation among ration components which were effective in these relations. Under the circumstances, it would be idle to attempt an analytical accounting for the differences in results obtained.

The foregoing observations having to do with nutrition on a production level, similar comparisons will be made of the values of the basal rations, fed on planes of approximate energy equilibrium. (Table 6.)

TABLE 6.—Metabolizable energy, heat increment, and net energy values of the basal rations, for maintenance

Period No.	Steer	Components of rations	Dry matter of daily ration	Metabolizable energy		Heat production, corrected	Fast-ing katabolism	Heat increment		Net energy per kilogram of dry matter	Utilization of metabolizable energy
				Total	Per kilo-gram of dry matter			Total	Per kilo-gram of dry matter		
			Grams	Calo-ries	Calo-ries	Calo-ries	Calo-ries	Calo-ries	Calo-ries	Calo-ries	Per cent
3.....	A	Oat straw, corn meal,	3,954	8,767	2,217	9,233	6,309	2,924	740	1,477	66.6
4.....	B	molasses.....	3,610	7,646	2,118	8,580	6,054	2,476	686	1,432	67.6
7.....	A	Timothy hay, corn	3,970	8,980	2,262	8,869	6,309	2,560	645	1,617	71.5
8.....	B	meal.....	3,428	7,410	2,162	7,854	6,054	1,800	525	1,637	75.7
11.....	A	Alfalfa hay, wheat	4,801	10,978	2,287	10,122	6,309	3,813	794	1,493	65.3
12.....	B	bran, linseed meal...	4,148	9,025	2,176	9,056	6,054	3,002	724	1,452	66.7

In spite of the obviously marked differences between the nutritive values of the three basal maintenance rations, their metabolizable

energy per kilogram of dry substance was approximately the same for the three; thus the metabolizable energy values for the ration of oat straw, corn meal, and molasses for steers A and B were 2,217 and 2,118 Calories, respectively; for the timothy-hay and corn-meal ration the corresponding values were 2,262 and 2,162 Calories; and for the ration of alfalfa hay, wheat bran, and linseed meal the values were 2,287 and 2,176 Calories.

The heat increments per kilogram of dry matter of ration also agreed closely. These values for steers A and B, respectively, were, for the oat-straw and corn-meal ration, 740 and 686 Calories; for the timothy-hay and corn-meal ration, 645 and 525 Calories; and for the alfalfa-hay, wheat-bran, and linseed-meal ration, 794 and 724 Calories.

The net energy values per kilogram of dry matter of these rations for maintenance were, for steers A and B, respectively, for the oat-straw and corn-meal ration, 1,477 and 1,432 Calories; for the timothy-hay and corn-meal ration, 1,617 and 1,637 Calories; and for the alfalfa-hay, wheat-bran, and linseed-meal ration, 1,493 and 1,452 Calories.

Further, the utilization of the metabolizable energy of these three basal maintenance rations by steers A and B, respectively, were, for the oat-straw and corn-meal ration, 66.6 and 67.6 per cent; for the timothy-hay and corn-meal ration, 71.5 and 75.7 per cent; and for the alfalfa-hay, wheat-bran, and linseed-meal ration, 65.3 and 66.7 per cent.

These data reveal the fact that the two steers were in remarkably close agreement in capacity to utilize the three rations, but that each of the rations exhibited a higher metabolizable energy, a higher heat increment, and a lower percentage utilization of the metabolizable energy for steer A than for steer B.

In the light of these observations it is highly probable that the net energy value of the corn meal, for body increase, for steer A in the unsuccessful period 13 would have been essentially the same as was determined for steer B in period 14, if the conditions governing the heat production in the former case had not got out of control.

It is regrettable that the diversity which characterizes net energy values of individual feeding stuffs can not be factored in such manner as exactly to separate the complex combinations of contributing influences, and to assign each to its cause or source; but the situation is so exceedingly complicated that the best that can be done at this time is to study these influences, one at a time, in specially devised and elaborately controlled investigations, and thus demonstrate their existence, and the ways in which and the prominence with which they affect results.

The general significance of the results of this study, therefore, is to tend to support the conclusion of Forbes (7) that foodstuffs express their characteristic net energy values only as they are components of nutritively complete rations; which is equivalent to the position that net energy values of individual feeding stuffs are not constants; also, in this light, such values seem not to be characterized by accuracy commensurate with the particularity, the laboriousness, and the expense of directly determining them.

Intensive research on the energy metabolism of farm animals may be fully justified, however, by the determination of principles of

general applicability; and such principles may be of immense service to economic interests, even though compromises are made in utilizing them which are limited, in degree, only by the desirability of the immediate economic results.

The practical consequences of this conclusion are discussed in a paper by Forbes (8).

#### SUMMARY

The associative or supplementing effects of feeding stuffs in combination were studied by respiration calorimetry in a duplicate series of observations with two 2-year-old steers as experimental subjects, the specific object of the investigation being to learn whether the net energy values of feeding stuffs are affected by the nutritive balance of the combinations in which they are fed during 28-day periods of experimentation—in other words, to learn whether net energy values of individual feeding stuffs are constants.

Energy-balance determinations were made with each of the steers during fast, at the energy-maintenance level with three rations of different character, and at a production level, the rations being the same as the three fed at the maintenance level, plus corn (maize) meal equivalent to half of the estimated energy-maintenance requirement.

The three basal rations differed widely as to protein, mineral, and presumably as to vitamin contents and therefore differed in their capacities to supplement corn meal.

Heat production was measured by direct calorimetry, checked by indirect measurement.

The apparent digestibility of the corn meal differed decidedly as affected by the other constituent feeding stuffs of the rations in which it was fed and therefore affected the net energy values determined.

The apparent metabolizable energy values of corn meal, fed on a production level, differed between 2,946 and 3,339 Calories per kilogram of dry matter in the case of one steer, and between 2,944 and 3,387 Calories in the case of the other, as affected by the rations in which the corn meal was fed.

The apparent net energy value of corn meal, fed on a production level, differed between 1,645 and 2,518 Calories per kilogram of dry matter, in the case of one steer, as affected by the rations in which the corn was fed.

The average of the three lowest determinations of the digestible crude protein maintenance requirement of these steers was 21.7 g per 100 kg of live weight per day.

The ratios of carbon to nitrogen in the urine were 1.2, 1.2, 1.3, and 1.3 to 1 on high-protein rations, and 3.6, 3.2, 3.2, 3.7, 3.3, 3.8, 3.9, and 4.0 to 1 on low-protein rations.

The results tend to support the conclusion of Forbes that feeding stuffs express their characteristic net energy values only as they are components of nutritively complete rations.

Individual foodstuffs, it seems, are susceptible of only superficial evaluation apart from the rations in which they are used.

It is necessary, therefore, to redirect the interest of the student and of the practical animal husbandman to energy values of rations as wholes, especially of nutritively balanced rations, rather than to such values of their constituent feeding stuffs.

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## FACTORS AFFECTING INFECTION OF WHEAT HEADS BY *GIBBERELLA SAUBINETII*<sup>1</sup>

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### INTRODUCTION

The major portion of the work upon which this report is based was carried on in 1920, 1923, and 1924 as part of a general study of the diseases of cereals caused by *Gibberella saubinetii* (Mont.) Sacc. and by certain species of *Fusarium*. The present paper describes inoculation experiments and histological studies of wheat scab, or head blight, caused by *G. saubinetii*, together with such macroscopic observations as have a direct bearing on the host-fungus relationship.

### WHEAT SCAB A FLORAL INFECTION

According to Palchevsky (6),<sup>3</sup> Woronin in 1889 observed that wheat kernels infected with the conidial stage of *Gibberella saubinetii*, which he called *Fusarium roseum* Lk., were considerably lighter in weight than normal kernels. Woronin did not, however, investigate the method of infection or the factors influencing the severity of the disease.

In 1891, Arthur (2) reported that wheat scab is a floral infection. He states that the spores of the fungus blow through the air and lodge on the delicate parts inside the flower, and that the fungus soon penetrates the kernel, sapping its life, and forms new spores, which may spread to other flowers throughout the field. Atanasoff (3) also concluded that infection was local rather than systemic.

### SYMPTOMS

Wheat scab, or head blight, is found throughout the wheat-growing areas of the Corn Belt. If conditions are favorable for its development and spread, the disease, by preventing the normal growth of infected kernels, may cause a marked reduction in yield; and, because of the metabolic processes of the fungus within the kernels, the grain produced will be of inferior quality. (Fig. 1, A.)

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<sup>2</sup> The writers wish to acknowledge their indebtedness to Eugene H. Herrling, of the Department of Plant Pathology, University of Wisconsin, for making a large number of the photomicrographs used in the paper.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 797.

The first symptom of infection within a spikelet may be a pinkish tint in the anthers protruding between the glumes, or a water-soaked area on a glume that first turns purplish brown and then loses its

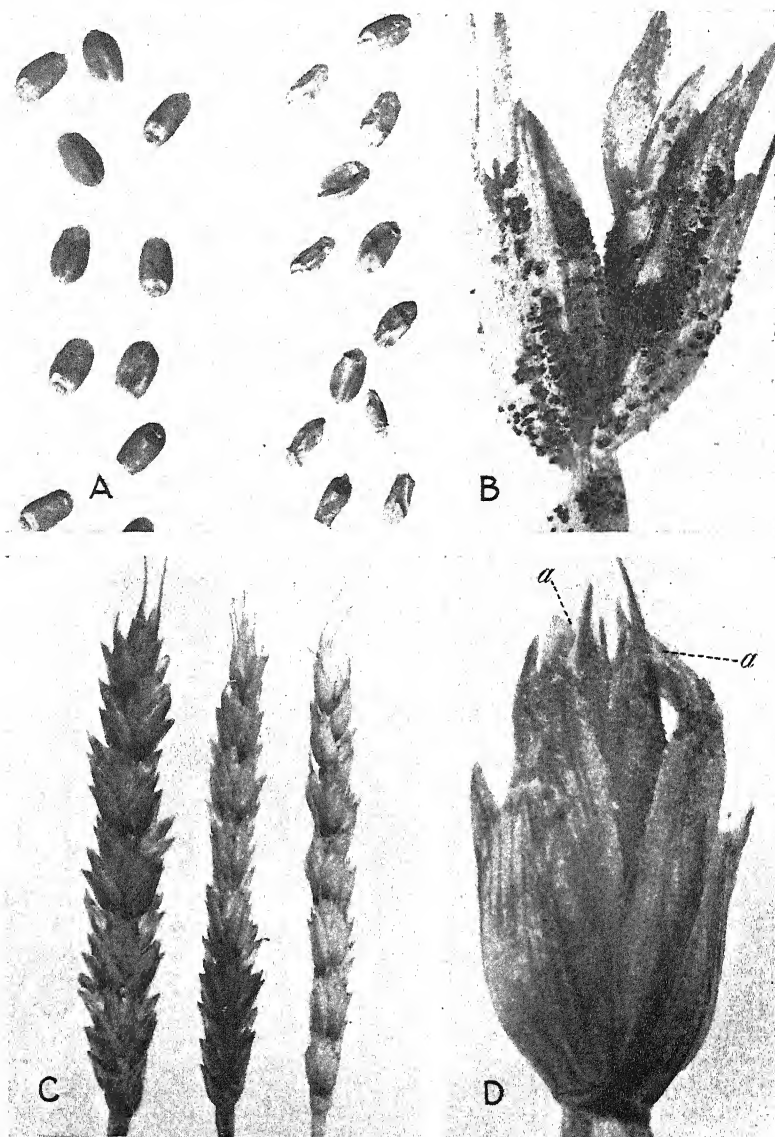


FIGURE 1.—A, Healthy kernels of wheat (left) and blighted or scabbed kernels (right); B, perithecia of *Gibberella saubinetii* on glumes and rachilla of infected spikelet of wheat; C, normal green head of wheat (left) and two infected heads (right), lower spikelets on middle head not diseased; D, infected spikelet containing caught anthers (a) and showing fungus growth on external surface of glumes

color. Later on, masses of conidia often give a pink color to the margins or surfaces of the glumes. (Fig. 1, D.) Small blue perithecia may occur on the glumes or at the base of spikelets, especially, in

certain bearded winter wheats. (Fig. 1, B.) If only scattered spikelets are infected, their smaller size and bleached glumes form a marked contrast to the adjacent normal green spikelets. The first infection usually occurs near the middle of the head. Spikelets just above and below those first attacked often develop the disease, and finally the entire head may be killed, as indicated by the characteristic bleached-straw color. (Fig. 1, C.)

#### MATERIAL AND METHODS

The experiments reported in this paper were carried out in the field and in greenhouses at the University of Wisconsin, Madison, Wis. Inoculations were made almost exclusively on pure-line Marquis and Prelude wheats. In the field, infections were studied as they occurred naturally and as they resulted from inoculations with a conidial suspension of *Gibberella saubinetii* on wheat heads in various stages of development, kept at the necessary high humidity in glassine bags during the period of incubation. In the greenhouse, plants in flats were brought to flowering by the aid of artificial light, so that they were ready for inoculation in the early spring. After inoculation the plants were placed in chambers in which the conditions of temperature and humidity could be controlled. The chambers were kept at constant temperatures of approximately 12°, 20°, 24°, 28°, and 32° C. At 28° and 32° the relative humidity was maintained at 70 to 75 percent. At the lower temperatures the humidity was lowered until the rate of evaporation, as indicated by the Livingston atmometer, was the same as that maintained at the higher temperatures. Notes were taken on the progress of infection until the disease had run its course or the heads were harvested.

The stages of development of the wheat heads recorded in the tables were determined by observation at the time of inoculation. Individual spikelets were recorded as "flowering" if one or more flowers displayed anthers that were not fully discharged. When the anthers were white and appeared to contain no pollen the spikelet was considered to be in the "past-flowering, A" stage. The period from the time when anthers no longer adhered externally to the glumes to the soft-dough stage was termed the "past-flowering, B" period in the development of the kernel.

The greater part of the histological material studied was killed in Gilson's fixative<sup>4</sup> or in Juel's zinc-chloride fixative,<sup>5</sup> embedded in paraffin, sectioned, and stained in Delafield's haematoxylin and erythrosin. The difficulty usually encountered in cutting serial sections of entire spikelets was overcome by demineralization with hydrofluoric acid. After being killed and partly dehydrated, entire spikelets as well as the hard mature kernels were immersed for 72 hours in equal parts of hydrofluoric acid and 70 per cent alcohol. For spikelets in the flowering stage, 53 hours' treatment with the hydrofluoric acid solution proved sufficient. The material was then washed in water for 24 hours and put through the paraffin-embedding process. Szombathy's "tanning" process, as recommended by Artschwager (9), was employed in fixing the paraffin ribbons to the slides.

<sup>4</sup> Gilson's fixative: 95 per cent alcohol, 126 cc; distilled water, 180 cc; glacial acetic acid, 54 cc; concentrated nitric acid, 6 cc; mercuric chloride (saturated solution), 33 cc.

<sup>5</sup> Juel's fixative: zinc chloride, 10 g; glacial acetic acid, 10 g; 50 per cent alcohol, 500 cc.

## FIELD AND GREENHOUSE STUDIES

## INITIAL INFECTION AND INCUBATION PERIOD

In the greenhouse and in the field, studies were made on initial infection and length of incubation period in wheat scab as influenced by temperature, humidity, and the stage of development of the wheat head.

In the greenhouse in 1923 and 1924, inoculation of the heads was more effective at flowering time than at earlier stages, though under certain conditions there was a relatively high percentage of infection

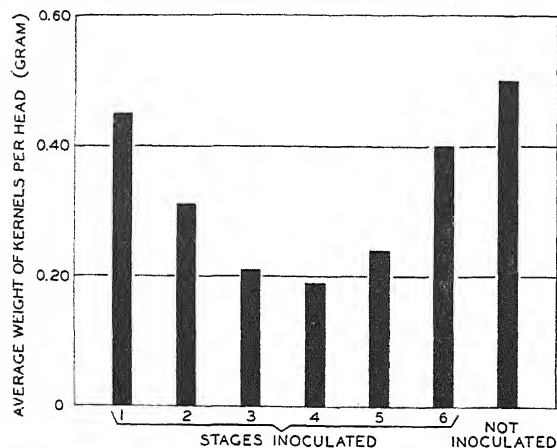


FIGURE 2.—Relation between stage of development in which heads were bagged and inoculated and the weight of grain per head. (Data in Table 2)

when the inoculum was applied in the out-of-the-boot and before-flowering stages. (Table 1.) In all cases it was necessary to maintain a high atmospheric humidity, without which infection is difficult. In the field, in 1924, inoculations made at flowering time or just previously gave the highest percentages of infected kernels per head. (Table 2.) Perhaps the best indication of the relation between the stage of development of the host and the severity of infection is the reduction in weight of infected kernels per head. Table 2 and Figure 2 show that the greatest reduction in weight of kernels occurred in heads inoculated at flowering time. The percentage of infection in both Marquis and Prelude wheat increased as the temperature rose. (Table 3.) At 12° C. infection occurred on extruded anthers but did not spread into the living tissues.

TABLE 1.—Infection in heads of wheat inoculated at various stages of development with *Gibberella saubinetii* and incubated during the progress of infection at 20°, 28°, and 32° C.

Stage of development at inoculation	Prelude wheat *			Marquis wheat		
	Heads inoculated	Heads infected		Heads inoculated	Heads infected	
		Number	Per cent		Number	Per cent
In boot.....	33	0	0	15	1	6.6
Half out of boot.....	13	0	0	17	1	5.8
Out of boot.....	14	0	0	51	31	60.7
Before flowering.....	52	14	26.9	100	55	55.0
Flowering.....	81	31	38.2	143	98	68.5

\* Notes taken for one week only because of injury by fumigation.

TABLE 2.—Average number of kernels, percentage of infected kernels, and percentage reduction in weight of kernels, per head, of Marquis wheat inoculated in the field at various stages of development with *Gibberella saubinetii*

Stage of development at inoculation	Healthy-kernel data		Infected-kernel data			
	Heads counted	Average kernels per head	Heads counted	Infected kernels per head	Heads weighed	Average reduction in weight of kernels per head following inoculation
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>
1. In boot.....	7	18.8	2	7.98	5	11.1
2. Emerging from boot.....	17	21.2	7	22.2	11	37.7
3. Half out of boot to before flowering.....	71	17.1	45	56.6	26	58.4
4. Flowering, A.....	15	19.9	11	50.0	17	63.0
5. Past flowering, A.....	16	23.0	11	43.24	15	52.9
6. Past flowering, B.....	30	23.8	19	35.2	31	20.4
Control (not inoculated or bagged).....	100	23.8	100	0	18	0

TABLE 3.—Infection in heads of Prelude and Marquis wheat inoculated at various stages of development with *Gibberella saubinetii* and incubated at various temperatures during the development of the disease

Temperature during incubation °C.	Prelude wheat			Marquis wheat		
	Heads inoculated	Heads infected		Heads inoculated	Heads infected	
	<i>Number</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Number</i>	<i>Per cent</i>
12.....	52	1	1.9	124	0	0
20.....	60	12	20.0	150	67	44.6
28.....	81	32	39.5	176	118	67.0
32.....						

In the greenhouse, the length of the incubation period was influenced by temperature, the higher temperatures accelerating the appearance of the disease. (Table 4.) The length of the incubation period varied also with the stage of development of the spikelet, decreasing as the plants matured, as shown by inoculations made July 16 to 25, 1924. (Table 5.) Although inoculum was applied in some instances as early as the out-of-the-boot stage, infection was not evident until the flowering stage. Such an interval implies either that infection may have occurred early but developed so slowly that it was not apparent until flowering time or that it actually did not occur before the flowering stage. Microscopic examination of sectioned material indicated that the latter supposition is correct. Moreover, under favorable conditions, the fungus may remain viable on the head for a number of days. In that case, the progress of infection on the wheat head might be expected to follow, to some extent at least, the progress of anthesis.

TABLE 4.—Incubation periods at various temperatures after inoculation of heads of Marquis wheat, in the before-flowering and flowering stages, with *Gibberella saubinetii*

Temperature °C.	Stage of development	Heads inocu- lated	Average length of incuba- tion period	Temperature °C.	Stage of development	Heads inocu- lated	Average length of incuba- tion period
		Number	Days			Number	Days
20.....	Before flowering.....	11	16.4	28.....	Flowering.....	16	8.1
20.....	Flowering.....	8	15.8	32.....	Before flowering.....	6	4.5
28.....	Before flowering.....	16	10.1	32.....	Flowering.....	21	4.5

TABLE 5.—Incubation periods after inoculation, in the field, of heads of Marquis wheat, in various stages of development, with *Gibberella saubinetii*

Stage of development	Heads inocu- lated	Average length of incuba- tion period	Stage of development	Heads inocu- lated	Average length of incuba- tion period
	Number	Days		Number	Days
Emerging from boot.....	18	a 12	Flowering.....	47	3.6
Half out of boot.....	40	6.9	Past flowering, A.....	25	a 5
Out of boot.....	35	6.6	Past flowering, B.....	35	3
Before flowering.....	28	4.1			

a Period probably too long. No reading for two days preceding the record of first infection.

#### ANTHESIS AND THE PROGRESS OF INFECTION

Concerning the interval between emergence from the boot and flowering, Percival says (7, p. 122, 124):

Anthesis, or opening of the flower, follows the escape of the ear from the upper leaf-sheath in five or six days, although in some instances it occurs on the first day, or may be delayed until the ninth day after the appearance of the ear \* \* \*. In ears possessing 16-18 spikelets the 8th to 12th usually open first; in those with 28-30, the 16th to 20th. Anthesis proceeds upwards and downwards from these points in more or less regular succession, the apical and basal spikelets being the last to flower.

According to Leighty and Sando (5, p. 236), it seems to be the consensus of opinion that the first spikelets to flower are situated in the upper part of the middle third of the head, or in the lower half of the upper third. They say that, considering the head as a whole, the flowers above the first flower to bloom complete their blossoming before those situated below this flower. In the spikelet, the lowest flower usually blooms first, followed by the others in order from lowest to highest on successive days. They also state (5, p. 240) that it seems evident—

That temperature, rainfall, and sunshine are intimately associated with the blooming of wheat flowers \* \* \*. Temperature, however, seems to be the most important of the meteorological influences affecting blooming.

In the experiments that they recorded, the maximum blooming occurred on May 15 and 16, days when the temperatures were 76° and 78° F., respectively, and when almost perfect sunshine with no rainfall was registered. Anthers in flowers opening on clear days dehiscid more rapidly than those in flowers opening on cloudy days. They state that—

Cloudiness or rain has the effect of retarding blooming, principally through the lowering of the temperature to a point below the optimum for this process. On cloudy or rainy days, however, when the temperature is favorable for blooming the flowers either open incompletely or bloom cleistogamously. On May 18 flowers were observed opening in a drizzling rain and pollen was shed from the anthers. No flowers were observed to open at any time during a rain of such intensity as to permit droplets of water to adhere to the glumes.

In 1923, experiments on 27 heads of Marquis wheat inoculated before blossoming by spraying with a conidial suspension of *Gibberella saubinetii* and incubated under controlled conditions of temperature and humidity showed that the location of first infections paralleled somewhat the theoretical progress of anthesis, as illustrated in the following tabulation:

Spikelet No. <sup>6</sup>	Number of first infections <sup>7</sup>	Spikelet No. <sup>6</sup>	Number of first infections <sup>7</sup>
1-----	0	7-----	6
2-----	0	8-----	3
3-----	0	9-----	6
4-----	1	10-----	1
5-----	4	11-----	3
6-----	8	12-----	3

In these inoculations, 77.1 per cent of the first infections were recorded as occurring on spikelets 5 to 9. No record was made of the average number of spikelets per head. The heads were, however, shorter than those grown in the field, which averaged a little more than 16 spikelets per head.

In 1924, inoculations were made in the field on heads of Marquis wheat that were flowering. Individual spikelets of these heads were recorded as "before flowering", "flowering", and "after flowering". The record of first infections on 25 of these heads showed 27 per cent of the first infections in spikelets that had not yet reached the flowering stage at the time of inoculation, 53 per cent in those that were in the flowering stage, and 18 per cent in those that were in the past flowering stage at the time of inoculation.

In 1923, heads of Marquis wheat in the early-flowering stage were inoculated with a conidial suspension of *Gibberella saubinetii* and incubated at 20° and 28° C. The results (Table 6) show that all first infections occurred on spikelets in flower or on spikelets adjacent to them.

On July 18, 1924, heads of Marquis wheat in the early-flowering stage were inoculated in the field with a conidial suspension of *Gibberella saubinetii*. The results (Table 7), with two exceptions, were similar to those obtained in the preceding experiment.

On July 19, 1924, heads of Marquis wheat in the flowering and past-flowering stages were inoculated in the field with a conidial suspension of *Gibberella saubinetii*. The results (Table 8) show that the position of first infection was less regular than in the two previous experiments. Because of the difficulty of determining the later stages in the development of the spikelets, errors are more apt to occur than in records of the earlier stages. Nevertheless, Table 8 indicates that first infections follow in some measure the progress of anthesis, since the larger percentage of first infections on the

<sup>6</sup> Spikelets are numbered from base of head upward.

<sup>7</sup> In some cases 2 adjoining spikelets on a head were found infected at the first reading.

lower spikelets occurred on the more mature heads. In Table 8 the percentage of infections occurring below spikelet 7 is 42.3; in Table 7, 20.

It is difficult to compare the progress of infection on wheat heads with the progress of anthesis because both are often irregular. Apparently there are numerous exceptions to the general order of progression in flowering upward and downward from some spikelet in the upper part of the middle third of the head. According to Percival (7, p. 124), "Sometimes 4 or 5 spikelets on one side open before the rest; in other cases adjacent spikelets on opposite sides of the rachis flower almost simultaneously." Data on the progress of infection by spikelets on heads inoculated before blossoming and incubated at 20° and 28° C. are given in Tables 9 and 10. It will be noted that in some cases the progress of infection seems to parallel the theoretical progress of anthesis. In other cases the relationship is not so evident.

In order to learn the trend of infection, two composite heads, A and B, were constructed from Tables 9 and 10, respectively. The resulting summaries are given in Table 11.

In head A infection first appeared in spikelet 7 and spread upward and downward, the basal spikelet being the last to show infection. The time between the appearance of the first infection and the infection of the basal spikelet was 8.5 days.

In head B infection first appeared in spikelet 9, and in 4 days spread upward to spikelet 15 and downward to spikelet 4. Heads 362 and 410, of Table 10, were irregular from the standpoint of the theoretical progress of anthesis. As a result, infection appeared in the first three spikelets of head B, before it appeared in spikelet 4. Otherwise the progress of infection upward and downward from the initial infection followed the theoretical order of anthesis. At 28° C. the time between the first infection and the last infection on head B was 4 days. This period, compared with the 8.5 days at 20° required to complete the spread of infection in head A, corresponds with the probable difference in the rate of flowering at the two temperatures. Concerning the time required for flowering, Percival (7, p. 124, 128) says:

At Reading the whole ear often completes its flowering in 3 to 5 days when the air is warm and the sky clear; in wet or dull weather the period is prolonged to 6 or 8 days.

In view of the possibility that infection may proceed through the rachis from the spikelets first infected to adjoining spikelets, a study was made of serial sections of the rachis on either side of visibly infected spikelets and also of serial sections of apparently clean spikelets adjoining spikelets visibly infected at the time of fixation. Mycelium was found to have advanced from infected spikelets through the rachis for some distance in both directions, but in the sections examined it had not actually invaded the adjoining spikelets. Evidently this rachis infection usually resulted in cutting off the food and water supply of spikelets as well as in producing toxic by-products, thus bleaching the spikelets and shriveling the kernels. These symptoms may easily be confused with those produced by the presence of mycelium within the spikelets. Often the real condition can be determined only by plating the kernels, or by microscopic ex-



amination of sectioned material. Undoubtedly some of the spikelets recorded as infected in Tables 7, 8, 9, 10, and 11 may be of this type. It is also conceivable that long intervals between initial infection and infection of neighboring spikelets may indicate infection through the rachis rather than from the exterior.

TABLE 6.—Position of spikelets showing first infections in heads of Marquis wheat inoculated in the early-flowering stage with *Gibberella saubinetii* and incubated at 20° and 28° C.

[F denotes flowering stage; asterisk (\*), first infection]

Temperature (° C.)	Head No.	Spikelet Nos. <sup>a</sup> flowering when inoculated and those showing first infections														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
20.	360					F		F		F	F	F*				
20.	361					F*	F*	F	F*	F*	F	F	F	F		
20.	465							F*	F*							
20.	468							F	F	F*	F*					
20.	471				F	F*	F	F	F	F	F*	F	F	F		
28.	365				F	F	F	F	F	F	F	F*	F			
28.	368				F	F	F	F	F*	F	F	F	F			
28.	369						F*									
28.	433					F	F	F	F	F	F	F	(*)			
28.	439				F	F	F	F*	F	F	F	F	F	F	F	
28.	440					F*	F	F	F	F	F	F	F	F	F	F
28.	445						F	F	F	F	F	F*				
28.	450						F	F	F	F*	F	F	F	F		
28.	451						F	F	F	F	F	F*	F*			
28.	460					(*)	F	F	F	F	F	F*	(*)			

<sup>a</sup> Spikelets are numbered from base of head upward.

TABLE 7.—Position of spikelets showing first infections in heads of Marquis wheat inoculated in the field, in the early-flowering stage, with *Gibberella saubinetii*

[F denotes flowering stage; asterisk (\*), first infection]

Head No.	Spikelet Nos. <sup>a</sup> flowering when inoculated and those showing first infections														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
584.								F*	F	F	F	F	(*)		
586.							F	F	F	F	F*	F			
587.							F	F	F	F	F	F	(*)		
588.								F	F	F	(*)				
589.								F*	F	F	F	F			
590.						F*	F*	F	F*	F	F	F			
591.						(*)	F	F	F	F	F	F			
592.						(*)	F	F*	F	F	F	F			
593.						(*)	F	F	F	F	F	F			
594.						(*)	F*	F	F	F	F	F	F	F	
595.							F	F*	F	F*					
596.								F*	F	F					
598.						(*)	F	F*	F	F	F	F	F		
599.							F	F*	F	F	F	F	F		
601.							(*)	F	F	F	F	F			
602.							(*)	F*	F	F	F	F	(*)		
603.			(*)					F	F	F	F	F	F		
604.							F	F*	F	F	F	F			
605.							F*	F*	F*	F	F	F			
606.							F	F*	F	F	F	F			
607.					F	F	F	F*	F	F	F	F	F	F	
608.								F	F	F	F*	F	F		
609.						F*	F	F	F	F	F	(*)			
610.			(*)				F	F	F	F	F				
611.							F	F*	F	F	F*				

<sup>a</sup> Spikelets are numbered from base of head upward.

TABLE 8.—Position of spikelets showing first infections in heads of Marquis wheat containing spikelets in the before-flowering, flowering, and past-flowering stages when inoculated in the field with *Gibberella saubinetii*

[F denotes flowering stage; P, past-flowering stage; asterisk (\*), first infection]

Head No.	Spikelet Nos. <sup>a</sup> flowering and past flowering when inoculated and those showing first infections																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
622			F				P	P*	F	P		P	P	F	P	F	P
624					F	F	F*	F	P	F*	P	P					
626					F	F	F	F	F	F	P	P		P			
628						F	F	F	F	F	P	P	F	F*	F	F	F
630							P	P	P*	P*	P	P					
632							F	F	F	F	P	P					
633				(*)			F	F	F	F	P	P					
634				(*)	P	P		P	P	P	P	P					
636				F*	P	P		P*	P	P	P	P	F	P		P	
637				P	P	P		P*	P	P	P	P					
638				F	P	P		P*	P	P	P	P			P		
639			(*)	(*)		P	P	P	P	P	P	P	F				
640						F	P	P	P*	F	F	P	P	F	P		
641						F	F	F	F	F	P	P	P	F			
642				(*)	F	F*	F	F*	F	P	P	P	F	P			

<sup>a</sup> Spikelets are numbered from base of head upward.

TABLE 9.—Progress of infection by spikelets on heads of Marquis wheat inoculated before flowering with *Gibberella saubinetii* and incubated at 20° C.

[Asterisk (\*) denotes first infection]

Stage of development when inoculated	Head No.	Position of first infection and interval (in days) between first and later infections for spikelet No.—												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Out of boot	388						4	4	2	(*)	2	4		
	391						9	8	8	8	8	(*)		
	398	8	8	8	7	8	(*)	7	5					
	399	5	5	5	4	5	(*)	7	5					
	406	2	2	2	(*)	(*)								
Before flowering	493	8	8	8	7	7	5	(*)	5	7	7	7	7	
	498			2	2	(*)	2	2						

<sup>a</sup> Spikelets are numbered from base of head upward.

TABLE 10.—Progress of infection by spikelets on heads of Marquis wheat inoculated in the out-of-the-boot stage with *Gibberella saubinetii* and incubated at 28° C.

[Asterisk (\*) denotes first infection]

Head No.	Position of first infections and interval (in days) between first and later infections for spikelet No.—														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
362					2				3	2	(*)	(*)			
366						(*)									
367						2	2	1	(*)	(*)	1	2	2	2	
385					5	5	4	1	3	(*)	3	3	3		
410	2	2	2	2	1	1				2	1	(*)			
411	4	4	4	3	(*)	2	3								
413	3	3	3	3	1	(*)	1			3	4				
428						4	3	3	2	2	(*)	(*)	3		
429						2	2	2	(*)						
430					3	3	(*)	3	1	3	3	3			

<sup>a</sup> Spikelets are numbered from base of head upward.

TABLE 11.—Progress of infection by spikelets on two composite heads <sup>a</sup> of Marquis wheat

[Asterisk (\*) denotes first infection]

Composite head	Temperature (° C.)	Position of first infections and interval (in days) between first and later infections for spikelet No. —														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A-----	20	8.5	7.5	7.2	5.7	4.9	4.3	(*)	4.2	4.2	7	7	7			
B-----	28	2	1	2	4	3.8	3.5	2.4	2.1	(*)	2.2	2.0	2.7	2.7	3	4

<sup>a</sup> Method of construction of composite head A: The position of the spikelet in which first infection would occur in the composite head was determined by multiplying the number of times first infection occurred on each spikelet by the position number of that spikelet; the sum of these products divided by the total number of first infections gave the location of initial infection in the composite head as 6.63, or spikelet 7. The heads in Table 9 were then shifted in such a manner that all first infections fell in the seventh spikelet position, and the mean interval of time between the first and later infections was determined for each spikelet position.

<sup>b</sup> Spikelets are numbered from base of head upward.

A possible explanation of the occurrence of infection at blossoming time was suggested by Atanasoff (4, p. 90). He says that "Fusaria can not infect the cereal crops before the plants have passed the blossoming period, i. e., before they cease rapid development and cell division." It is characteristic of weak parasites that they are more destructive when the host is growing under unfavorable conditions. Adams (1, p. 116-117) mentions this point in relation to head blight and cites several examples. In the present investigation the plants were kept under as favorable growing conditions as possible, except that in some cases in the greenhouse unfavorable temperatures were used. At the higher temperatures the percentage of infection reached its maximum. With the exception of the single head of Prelude wheat found infected at 20° C., which was recorded as inoculated before flowering, the highest percentage of infected heads at all temperatures was found in the groups that were flowering when inoculated. The low percentages of infection in Prelude wheat as compared with Marquis (Table 1) are due, partly at least, to the fact that notes on Prelude were taken for only 1 week after inoculation, whereas notes were taken on Marquis until the plants were dead, and also to the fact that Prelude is more resistant than Marquis, as indicated by later experiments.

No infection occurred at 12° C., a temperature decidedly unfavorable for the rapid development, pollination, and fertilization of wheat plants when in the flowering stage. Threshing notes show that practically no kernels had been set on plants grown at that temperature. Leighty and Sando (5, p. 242) report that flowers were not seen to open at a temperature below 56° F. (13° C.), but that pollen was observed to be discharged at 52° F. (11° C.). Authorities cited by them give 12°, 13°, 14°, and 16° C. as the minimum temperatures at which flowers were observed to open. Some of the plants used in these experiments were flowering when they were inoculated and placed in the 12° chamber, so that certain spikelets may be considered to have flowered normally. Nevertheless infection failed to occur, probably because of the effect of an unfavorable temperature on both host and fungus, and possibly because of a lack of adequate humidity in the low-temperature chamber.

It seems clear that infection does not usually occur first on the outside of the glumes. Only one spikelet of the many examined showed the parasite growing on the outside of the enveloping glumes. In that case it appeared to be growing in a drop of water lodged on the surface and had not actually penetrated the glume. No fungus was found within the spikelet. Inoculation experiments in the field in 1924 tend to confirm the belief that infection does not originate on the outside of the glumes. Areas on the outside near the center of 30 glumes on spikelets in the before-flowering to the just-past-flowering stages were marked on 18 heads with a ring of india ink and then inoculated by applying a drop of spore suspension in sterile water, or a bit of infected agar, or by pricking with a needle dipped in spore suspension. The material was fixed at intervals of from 1 to 9 days. Macroscopic observations indicated that no infection had occurred except in the case of the needle-prick inoculations, although conditions of humidity and temperature were favorable for rapid infection. The prick inoculations were made in a cool period with minimum and maximum temperatures of 14° and 18°, respectively, at which the rate of infection is usually slow. Of the 9 glumes cut, only 2 showed penetration by the fungus. In one of these the hyphae had not advanced beyond the cells injured by the needle prick; in the other, hyphae were found to have penetrated to the depth of a few cells at the base of a spikelet inoculated with infected agar.

Further indication that initial infection does not occur on the outside of the glumes was found upon examination of serial sections of a number of diseased spikelets. Although the kernels showed a network of mycelium, there was practically no fungus on the outside of the glumes.<sup>5</sup> When infection of a spikelet has developed sufficiently to attract the attention of a casual observer, there are often sporodochia at the base and along the margins of the outer glumes. The possibility that conidia may be held in drops of water at the base of the spikelet until invasion has been effected is recognized, although it has not been observed to occur in any of the numerous cases of initial infection studied. Growth of the fungus on the exterior seems to follow infection within the glumes.

After anthesis, infection appears to occur first on degenerating tissues such as anthers, styles (fig. 3, A and B), and pericarp, and to penetrate from these into the developing kernel or the inner surfaces of the glumes.

Caught and retained anthers were the first tissues observed to serve as a base for invasion into the developing kernel. In field inoculations during 1920 it was noted that anthers caught between glumes frequently showed the first sign of infection. Within a short time the glume from which such an anther extended developed a water-soaked and discolored area. By fixing and sectioning spikelets in the very early stages of infection, clear cases of infection beginning in caught and held anthers were found.

Little information has been found in the literature on the frequency of retained anthers. Leighty and Sando (5, p. 233) noted that 5 per cent of the 406 flowers on the heads under observation did not open at all or opened only far enough to allow the tips of the anthers to

<sup>5</sup> The term "glume" is here construed in the broader sense to include lemma and palea.



FIGURE 3.—A, Portion of a longitudinal section of a spikelet showing an early stage of anther infection; *h*, hyphae.  $\times 57$ . B, Portion of a degenerating style which apparently served as a connecting tissue between an infected anther and the kernel in the same flower.  $\times 136$ . C, Portion of a longitudinal section of the developing kernel in the flower containing the anther in A; serial sections show that there is no fungus in the kernel.  $\times 57$

extrude between the glumes, but that all these flowers set kernels. Percival (7, p. 124) says:

Retention of the anthers, which is most frequent in dense-eared wheats, occurs chiefly in the lower spikelets of ears and the smaller flowers of each spikelet, some of which do not open at all.

In the season of 1924 records were made of retained anthers in inoculated heads and also in the 100 uninoculated heads of Marquis wheat that were harvested for the purpose of obtaining information on caught anthers, independent of inoculation. Notes were taken only on the first and second flowers, for it was found upon examination of 15 heads of uninoculated Marquis wheat grown in the field that only 6 per cent of the third flowers in the spikelets had set kernels. The data presented in Table 12 show that there were retained anthers in 22.5 to 85.5 per cent of the fertile flowers observed and in 58 to 92 per cent of the spikelets counted. They also indicate that retention of

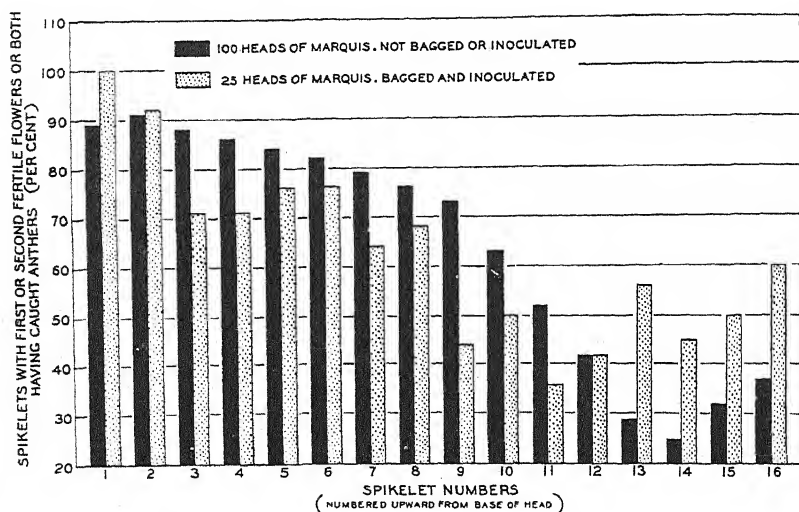


FIGURE 4.—Distribution of spikelets in Marquis wheat having one or both fertile flowers with caught anthers, based on 100 heads unbagged and uninoculated and 25 heads bagged and inoculated with *Gibberella saubinetii* in the field. (Data in Table 12)

anthers is not the result of infection, because the percentages for checks were higher than for inoculated plants. The data for Marquis wheat are presented graphically in Figure 4. It will be noted in Figure 4 that a comparatively high percentage of retained anthers was found in spikelets Nos. 2 to 12, inclusive. The tabulation on page 777 and Tables 6 to 11 likewise show that, with three exceptions, all the first infections recorded occurred below spikelet 13. Two varieties of wheat studied, Norka (C. I. No. 4377<sup>9</sup>) and Carina (C. I. No. 3756) showed marked differences in the percentage of fertile flowers with retained anthers. (Table 13.) Likewise there was a higher percentage of infected kernels in the variety having the higher percentage of retained anthers.

<sup>9</sup> C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

TABLE 12.—Percentage of anthers held by glumes after anthesis in several varieties of wheat, uninoculated or inoculated, artificially or naturally, with *Gibberella saubinetii*, in the field

Variety	Treatment	Heads observed	Fertile flowers with retained anthers	Spikelets with retained anthers in first or second or both fertile flowers
		Number	Per cent	Per cent
Marquis.....	(Not inoculated.....	100	46.8	60
	Inoculated.....	25		58
	Natural infection.....	6	52	
Several varieties.....	do.....	15	34.5	
	do.....	8	22.5	
Norka (C. I. No. 4377).....	(Not inoculated.....	15	55.7	76.7
	Inoculated.....	18	47	
Carina (C. I. No. 3756).....	(Not inoculated.....	15	85.5	91.9
	Inoculated.....	11	73	
Variety not known.....	(Not inoculated.....	15	69.8	83.4
	Inoculated.....	14	57.8	

TABLE 13.—Percentage of fertile flowers with retained anthers and of kernels infected in Norka and Carina wheat inoculated in the field, in flowering to past-flowering stages, with *Gibberella saubinetii*

Variety	C. I. No.	Heads examined	Fertile flowers with retained anthers	Kernels infected
		Number	Per cent	Per cent
Norka.....	4377	18	47	* 19.5
Carina.....	3756	11	73	40.1

\* Percentage based on 17 heads.

## HISTOLOGICAL STUDIES

In connection with the experiments in both field and greenhouse, histological studies of inoculated spikelets were made in order to determine the mode and course of infection in more detail than was possible from macroscopic observations alone. By means of serial sections through entire spikelets it was possible to follow the course of infection in the various parts of individual spikelets and to ascertain the presence or absence of retained anther tissue.

## INFECTION OF GLUMES

It seemed clear from macroscopic observation of diseased spikelets that it is unusual for infection to begin on the outside of the glumes and then proceed to the kernel. Moreover, the inoculation of the outer surface of the glumes, in the field in 1924, as stated previously, gave negative results.

As long as the flowers of a spikelet remain closed the spikelet appears to be effectively protected against infection by the thickened and suberized walls of the outer epidermis of the glumes. This protection is found in the highest degree in the outer epidermis of the outer glumes and to a lesser degree in the outer epidermis of the lemmas and paleas. The walls of the inner epidermis of the empty

glumes and of both flowering glumes were thickened toward the distal ends, the thickening extending considerably farther toward the base of the spikelet in the empty glumes than in the flowering glumes. As a rule, the walls of the epidermal cells of the glumes are thickened where they are exposed and are practically impenetrable. (Fig. 5, A.) Penetration of the less thickened walls of the inner epidermis of the glumes is not so difficult and apparently occurs frequently. (Fig. 5, B.)

The heaviest growth of mycelium in badly infected glumes, rachis, or culms is found in the chlorophyllous tissue. (Figs. 5, C, and 6, C.) Apparently the nutrient value of these cells is high and their thin walls offer little resistance to the fungus.

Sporodochia are often observed at the base of spikelets in advanced stages of infection. The stroma supporting them seems to be in the chlorophyllous tissue. Conidiophores emerge through the stomata at first (fig. 6, D), but with the massing of mycelium within the tissue they break through the epidermis elsewhere, frequently pushing outward between the lateral walls of the epidermal cells. (Fig. 6, A and B.) Sporodochia may extend upward from the base of the glumes, particularly along their lateral edges, but this growth also appears to follow internal infection. (Fig. 6, C and D.)

The vascular elements are not particularly attractive to *Gibberella*, although any bundle that lies in the path of the hyphae may be invaded. (Fig. 7, B.) The phloem shows much heavier infection than the xylem vessels. The thin cellulose walls of the phloem and companion cells are apparently penetrated without difficulty, and it seems reasonable to suppose that a rich medium is offered. There appears to be less fungus in the annular and spiral vessels than in the pitted ones. A rather uniform constriction of the hyphae as they pass through the walls of the pitted vessels indicates that passage is effected through the pits. The manner of penetration of the walls of the annular and spiral vessels was not determined. It seems probable that penetration is effected at some unthickened part of the wall.

In thoroughly infected glumes, mycelium may be found throughout the thick-walled cells of the ground parenchyma, but, owing to the thick lignified cell walls and the absence of cytoplasm, it is not massed as it is in the assimilating tissue. (Fig. 7, D.) These thick-walled lignified cells do not collapse even when heavily infected. The fungus apparently makes its way from cell to cell through the pits and not by penetrating any of the lignified parts.

The thin-walled ground parenchyma of the rachis and culm is composed of short-lived cells that are polygonal or rounded in cross section and have finely pitted walls. In this tissue the hyphae are found chiefly in the intercellular spaces. (Fig. 7, A.) Infrequently, the hyphae go directly through the cell walls, but the intercellular path, being the line of least resistance, is usually followed. In general it may be said that *Gibberella saubinetii* is both intercellular and intracellular in the various tissues of the host. The path of the fungus as it goes from cell to cell leads through pits or thin places in the walls. The attenuation of the hyphae to a threadlike thinness as they pass through the walls seems to be of common occurrence in all tissues. (Fig. 7, C.)



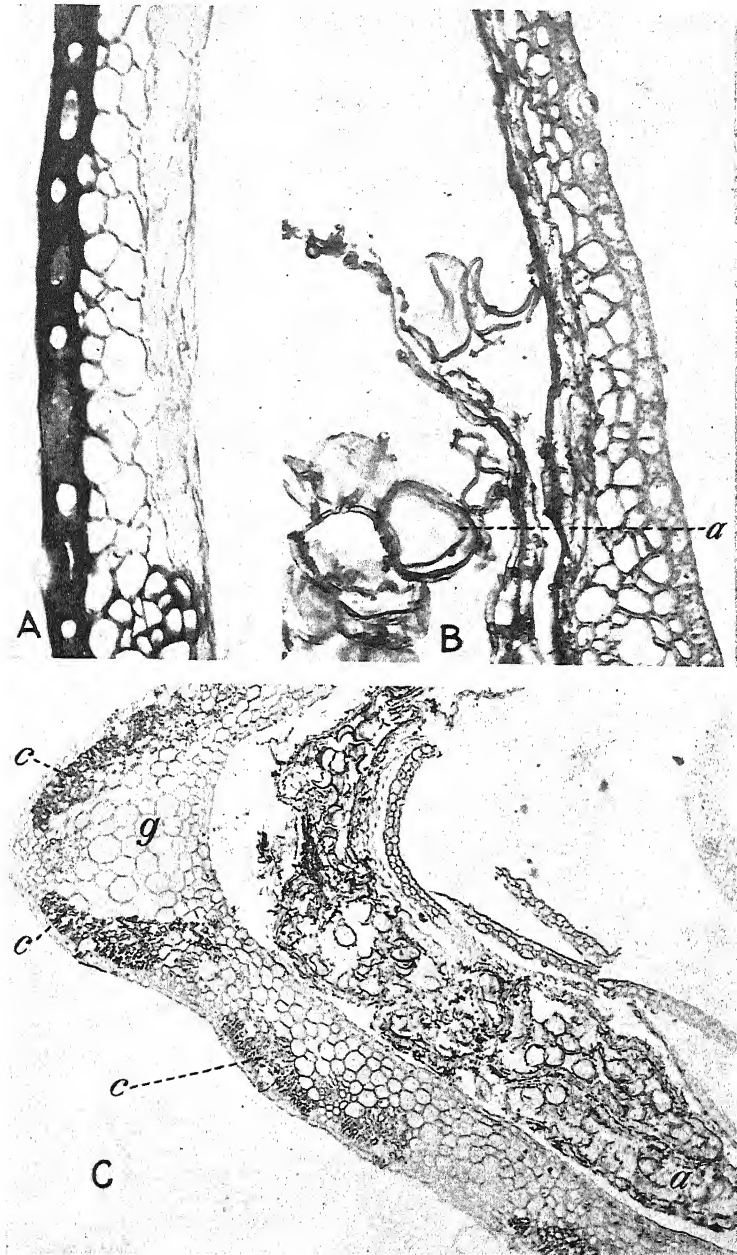


FIGURE 5.—A, Portion of a cross section of a lemma in an infected spikelet, stained with safranine and orange G to bring out differences in the nature and thickness of the walls of the epidermis on the outer and inner surfaces of the glume; the small amount of fungus present in the collapsed cells of the parenchyma is not brought out by these stains.  $\times 379$ . B, Portion of another cross section of the spikelet shown in A, stained with safranine, Delafield's haematoxylin, and orange G to show the advance of fungus hyphae from an infected anther (a) into the lemma.  $\times 265$ . C, Portion of a cross section of a spikelet, stained with Delafield's haematoxylin and erythrosin, showing location of chlorophyllous tissue (c) in an outer glume (g); caught anther (a) thoroughly permeated by hyphae; little if any fungus in glume.  $\times 55$

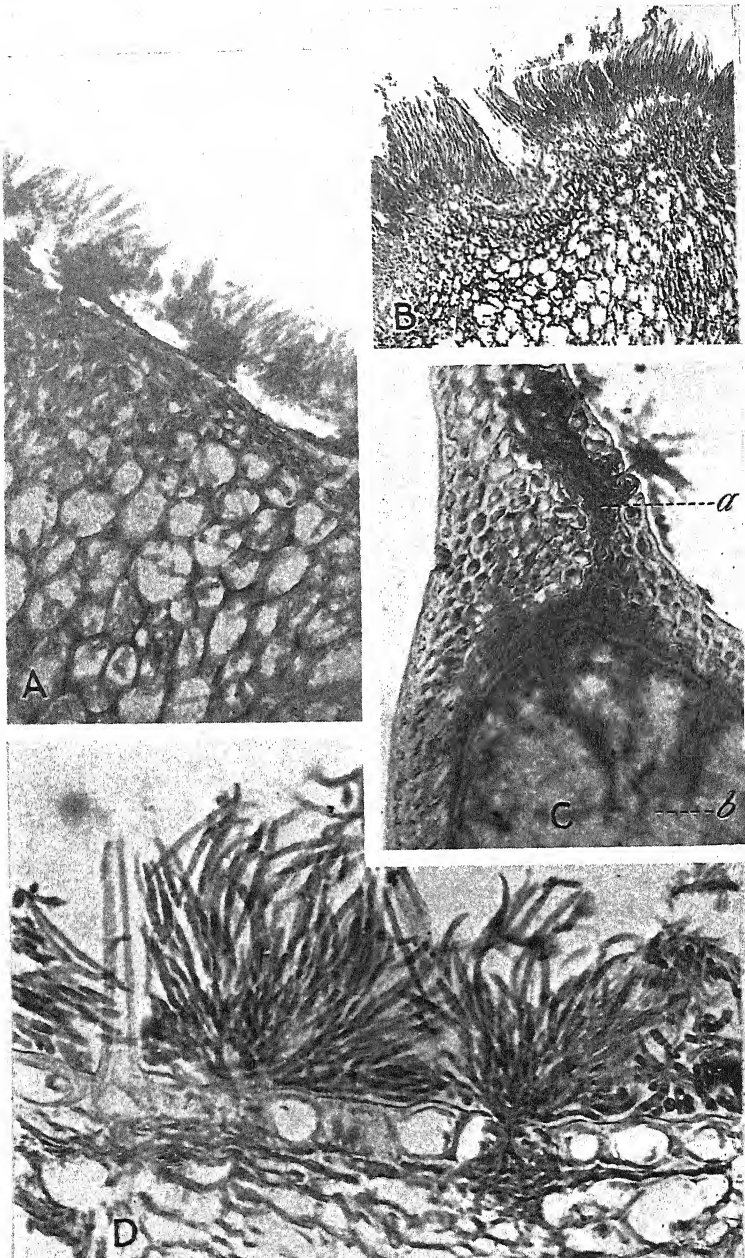


FIGURE 6.—A, Portion of a cross section through the rachis at the base of an outer glume, stained with Delafield's haematoxylin and erythrosin, showing hyphae emerging between cells of the epidermis and sporodochia on the surface.  $\times 91$ . B, Sporodochia at the base of a glume, stained with Delafield's haematoxylin and erythrosin; the fungus is well established in the chlorophyllous tissue and has emerged through the stomata.  $\times 194$ . C, Portion of a cross section of an infected palet showing entrance of the fungus through the inner epidermis, massing of hyphae in the chlorophyllous cells (a), and emergence through a stoma in the outer epidermis; the space (b) between the palet and kernel is webbed with hyphae.  $\times 194$ . D, Emergence of hyphae through the stomata and formation of sporodochia on the outer surface of the glume, stained with Delafield's haematoxylin and erythrosin.  $\times 347$

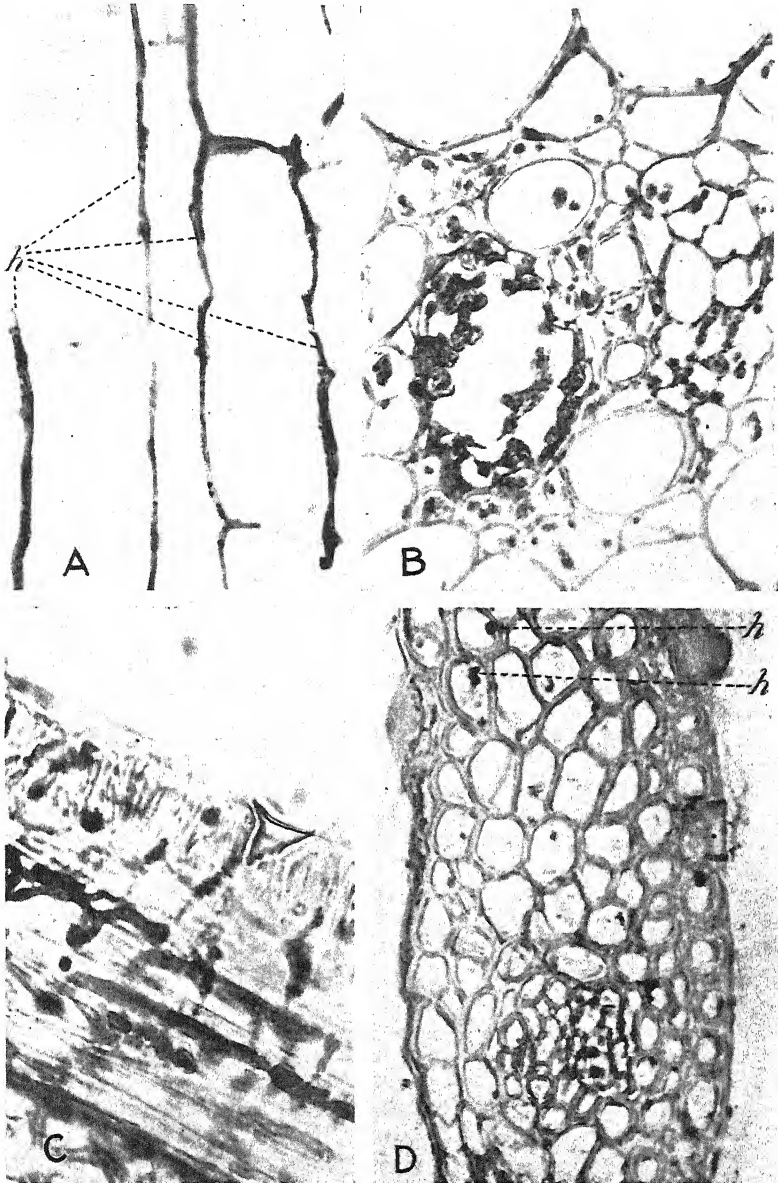


FIGURE 7.—A, Portion of a longitudinal section through the parenchyma of an infected rachis, showing intercellular path followed by the *Gibberella* hyphae. About  $\times 305$ . B, Portion of a cross section of an infected rachis, showing hyphae within the vascular bundle.  $\times 420$ . C, Portion of a longitudinal section of a lemma, showing the threadlike constrictions of the hyphae as they pass from cell to cell.  $\times 612$ . D, Portion of a lemma showing occasional hyphae (*h*) within the cells of the thick-walled ground parenchyma.  $\times 436$ . All sections stained with Delafield's haematoxylin and erythrosin.

## INFECTION OF ANTHERS

From a microscopic examination of fixed and sectioned material, it seemed evident that infection frequently begins in caught anthers. A number of spikelets fixed in the early stages of infection showed mycelium only within caught anther tissue. Others showed hyphae extending from infected anthers to adjacent parts of the flower. Of the material fixed and sectioned in 1920, 70 per cent of the 46 inoculated pieces either included infected anther tissue or contained no anther tissue and were not infected. Of the 1923 material, 29 of the 37 spikelets, or 78 per cent, of Prelude and Marquis wheat selected from the greenhouse inoculation experiments included either first or second flowers, or both first and second flowers, with retained anthers. Few cases of early infection were obtained in 1923. Serial sections of 8 spikelets, recorded at the time they were fixed as probable examples of infection originating in retained anthers, showed anther tissue in all, but only 4 were fixed early enough to show that invasion began in the anthers. (Fig. 3, A and C.) Anthers removed from a flower, inoculated with *Gibberella saubinetii*, incubated in a Van Tiegham cell at room temperature, and fixed 17 hours later, were found to be completely interlaced with the fungus hyphae (Fig. 8, B.)

Six good examples of initial infection through caught anthers, following the usual method of inoculation, were found among the 1924 spikelets sectioned. Additional information on this point was obtained in a special inoculation experiment in the field, in 1924, on the extruded anthers of 11 marked spikelets on 3 heads of Marquis wheat in the just-past-flowering stage. The extruded anthers were inoculated with a spore suspension of *Gibberella saubinetii* on July 30 and fixed on August 11. At the time of fixation, 8 of the spikelets appeared to be badly infected and 3 showed no symptoms of infection. None of the uninoculated spikelets showed infection. Two of the visibly infected spikelets were fixed and cut. Both contained caught anthers and kernels badly infected. It does not follow, however, that because one kernel in a spikelet is infected all are diseased. (Figs. 8 A, and 9, A-D.) Figure 10, A and B, shows cross sections through an infected anther of the second flower of a spikelet of Prelude wheat inoculated at flowering and fixed after an incubation period of 45 hours at 32° C. The serial sections show that the anther in contact with the kernel at the brush end is permeated by fungus and that hyphae have advanced into the pericarp of the second kernel. Toward the embryo end of this kernel the amount of fungus is scant. Hyphae have also penetrated the inner surfaces of the lemma and palea of the second flower, the outer surfaces in each case being clean, for the thickened and cutinized walls of the epidermis on the outer surface of the glumes render penetration difficult. Serial cross sections also show that this spikelet contains a basal and a third kernel, neither of which is infected. In the third flower there is a bit of infected caught-anther tissue, but hyphae had not reached any other part of the flower at the time the spikelet was fixed.

When infection occurs at flowering time, the fungus may easily penetrate the ovary. Shortly after anthesis the parenchyma of the pericarp begins to break down, the nuclei and cytoplasm of the cells disappear, the transition starch is moved into the developing seed, and the walls of the cells finally break down and are crushed together

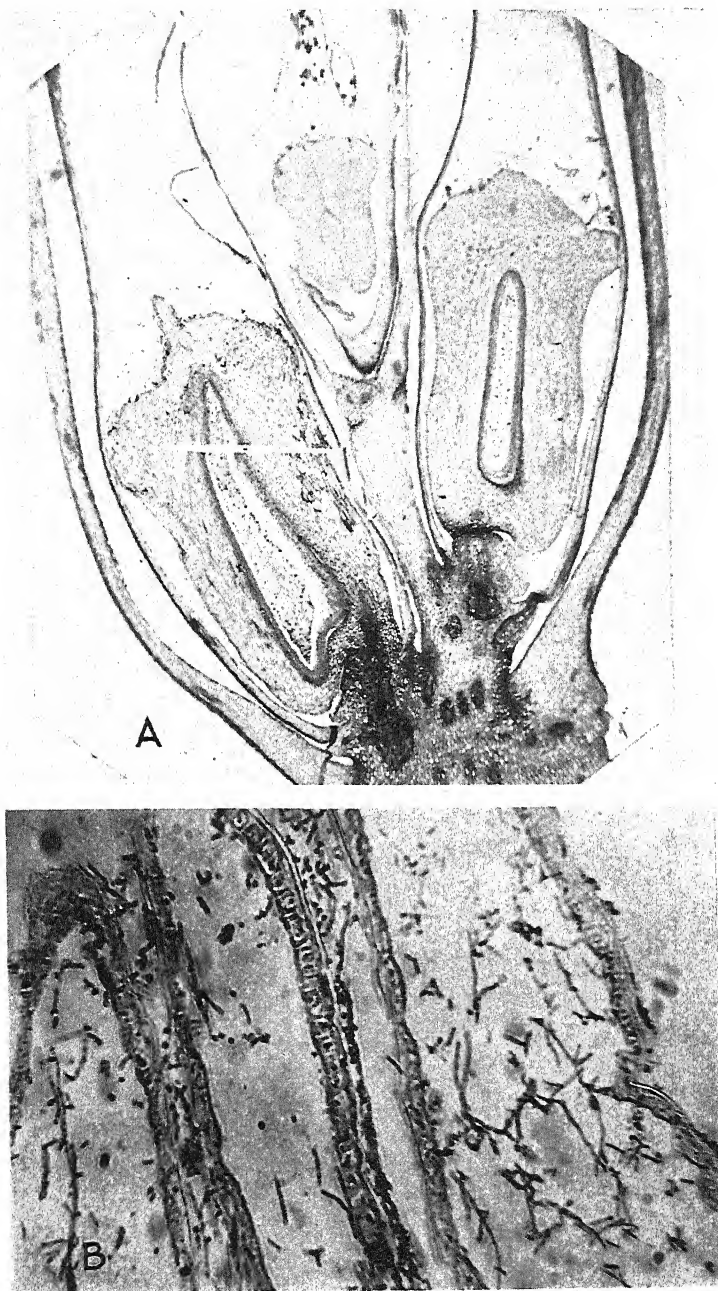


FIGURE 8.—A, Portion of a longitudinal section through an infected wheat spikelet. Serial sections of this spikelet show that the basal flower contains an infected caught anther and an infected kernel; the second flower is without a retained anther and the kernel is not infected; neither the anther tissue nor the kernel of the third flower is infected.  $\times 16$ . (Details shown in fig. 9.) B, Portion of a section of an anther inoculated with *Gibberella saubinetii* and fixed after 17 hours' incubation in a Van Tieghem cell.  $\times 142$ . Stained with Delafield's haematoxylin and erythrosin



FIGURE 9.—Portions of a longitudinal section through an infected spikelet. A, Uninfected anther retained in the third flower.  $\times 46$ . B, Developing kernel in the third flower. Serial sections show no hyphae in this kernel.  $\times 46$ . C, Infected caught anther (a) in the basal flower of the spikelet; g, Glume.  $\times 50$ . D, Infected basal kernel; p, pericarp (ovary); e, endosperm; s, remains of the style.  $\times 50$ . Stained with Delafield's haematoxylin and erythrosin. (Another section through this spikelet is shown in fig. 8)



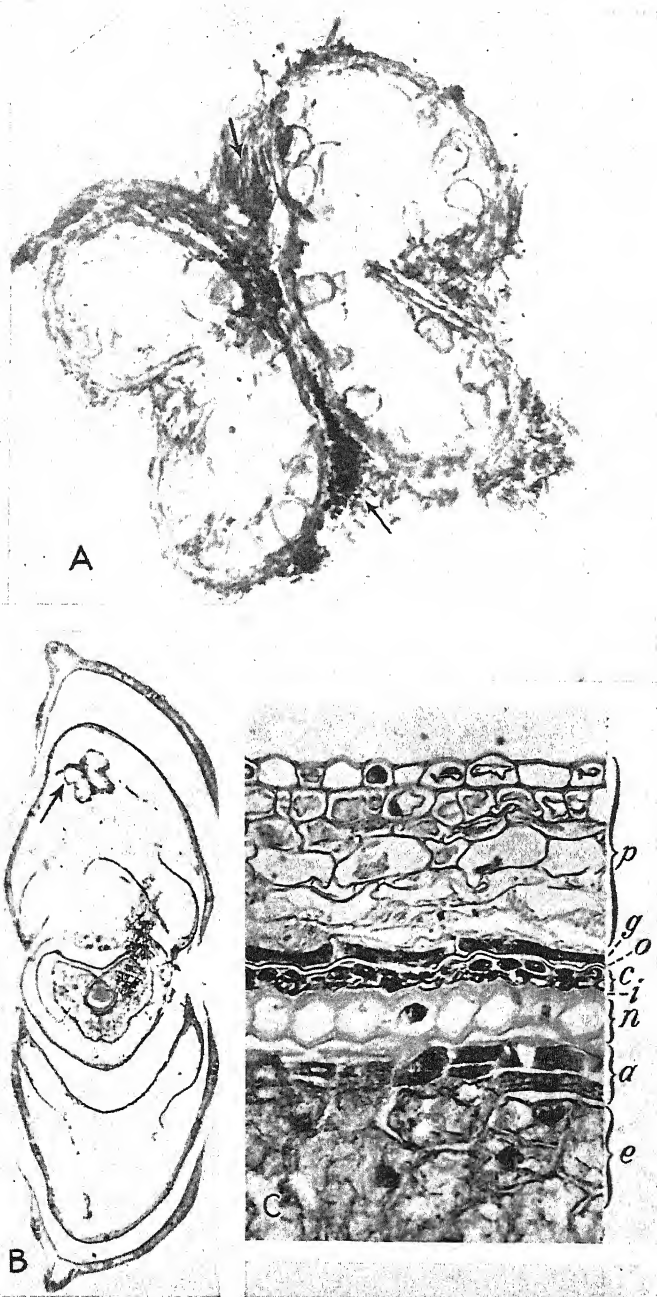


FIGURE 10.—A, Detail of anther, shown in B. Sporodochia containing conidia are indicated by arrows.  $\times 132$ . B, Cross section through the upper part of a spikelet, showing the presence of an infected anther within the glume.  $\times 12$ . C, Cross section of the outer layers of a developing kernel: *p*, Pericarp; *g*, chlorophyll layer (cross layer); *o*, outer layer of the testa (semipermeable membrane); *c*, color layer of testa; *i*, inner membrane of testa; *n*, epidermis of nucellus; *a*, aleurone cells forming; *e*, endosperm.  $\times 264$ . Stained with Delafield's haematoxylin and erythrosin

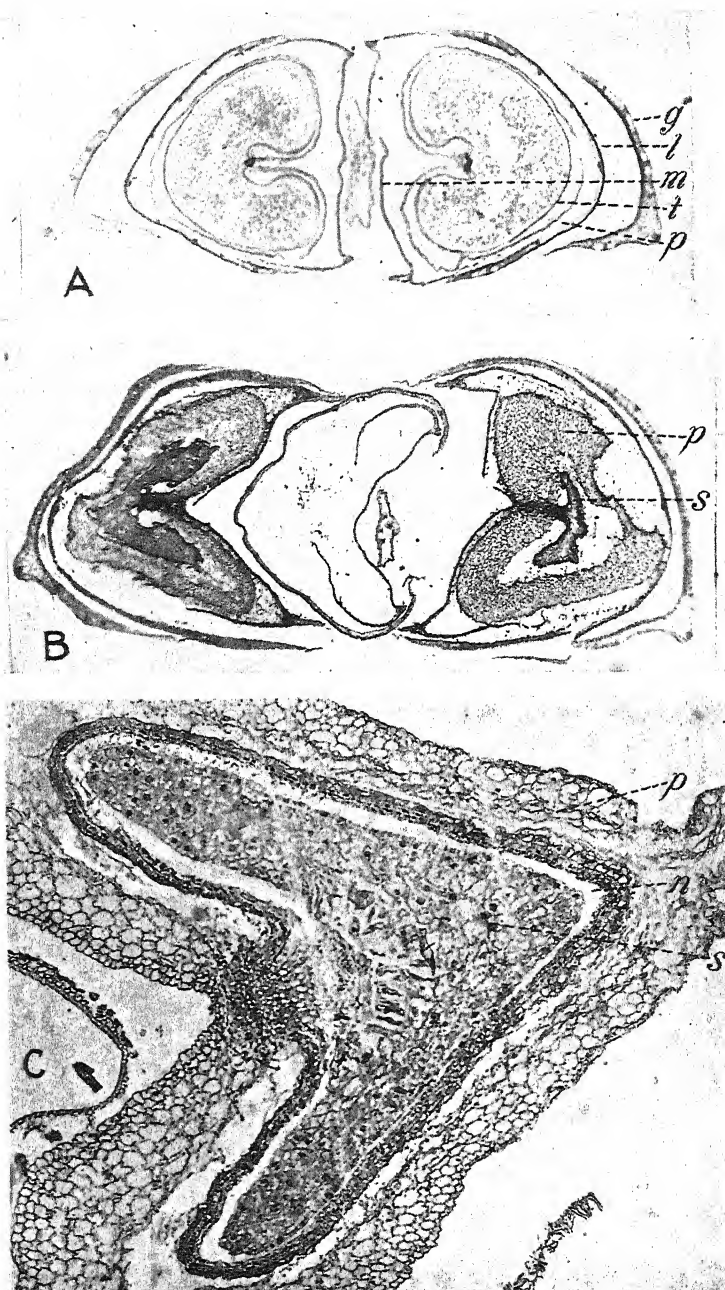


FIGURE 11.—A, Cross section of a nearly mature uninfected spikelet of wheat: *g*, Outer glume; *l*, lemma; *m*, palea; *p*, pericarp; *t*, testa.  $\times 68$ . Stained with Flemming's triple stain. B, Cross section of a spikelet in which both kernels are badly infected: *p*, Pericarp; *s*, seed.  $\times 85$ . Stained with Delafield's haematoxylin and erythrosin. C, Cross section of an immature kernel showing the relation of the various structures at that stage of development. The cells of the ovary wall (*p*, pericarp) are degenerating, and the developing seed (*s*) has almost filled the nucellar space (*n*).  $\times 277$



in the growth of the kernel. (Figs. 10, C, and 11, C.) If entrance into this tissue is effected early, the fungus advances along the intercellular spaces or from cell to cell without resistance, permeating the entire kernel (fig. 11, B). Later, the pericarp is less easily penetrated and is not so well supplied with nutrient substances as at flowering time (fig. 11, A). As the kernel matures, the layers of the testa also become a barrier to the fungus. (Figs. 10, C, and 12.) The layers of

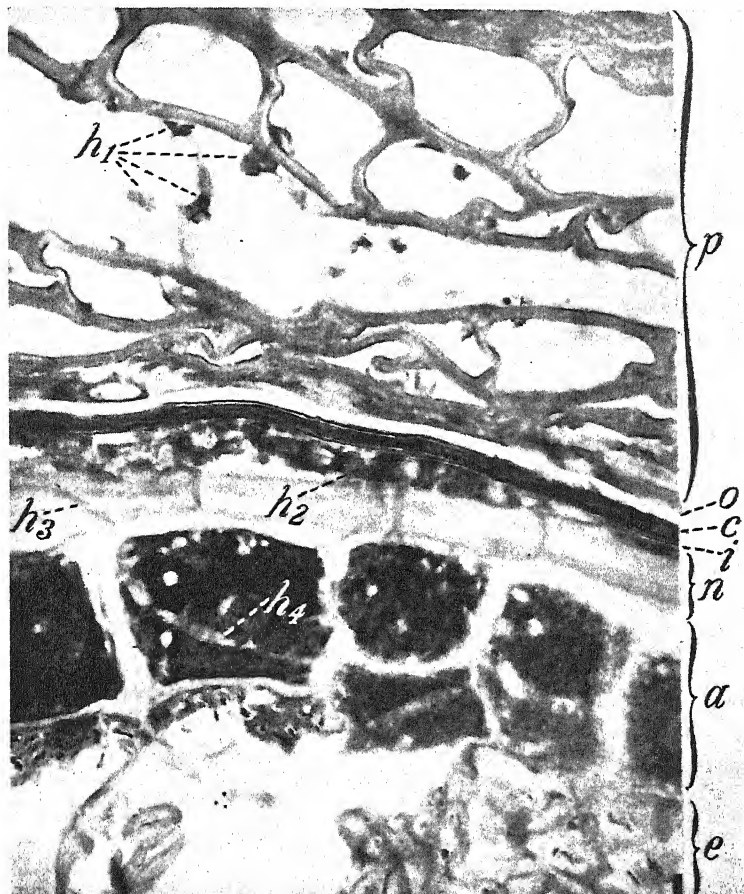


FIGURE 12.—Portion of a cross section through the outer layers of a mature wheat kernel: *p*, Pericarp; *o*, outer layer of testa (semipermeable membrane); *c*, color layer of testa; *i*, inner membrane of testa; *n*, epidermis of nucellus; *a*, aleurone cells; *e*, endosperm; *h*<sub>1</sub>-*h*<sub>4</sub>, hyphae.  $\times 600$ . Stained with Delafield's haematoxylin and erythrosin

the testa appear to be more resistant to hyphal penetration than other parts of the kernel. In figure 12 hyphae can be seen in the pericarp (*h*<sub>1</sub>), separating the inner membrane of the testa from the nucellar layer (*h*<sub>2</sub>), passing through the nucellar layer (*h*<sub>3</sub>), and invading the aleurone cells (*h*<sub>4</sub>), but, in this portion of the section, nowhere penetrating the testa. In late infections the kernel is not permeated throughout by hyphae; whatever fungus enters the kernels is more

or less confined to the pericarp at the embryo end and to the embryo itself (8).

It is impossible to say how often infection originates in caught anthers, nor can it be said that infection never occurs except by the contact method with anthers or other degenerating tissues that serve as saprophytic media for early growth of the fungus. This method, however, seemed to be the rule under the conditions of the experiments described. Infection was most frequently observed to begin in degenerating anther tissue, which apparently furnishes an ideal medium for the rapid growth of *Gibberella saubinetii* under favorable conditions of temperature and moisture.

### SUMMARY

Data presented in this paper are based upon macroscopic observations of inoculations with *Gibberella saubinetii*, made in the field and greenhouse, on heads of pure-line Prelude and Marquis wheat, and upon a microscopic study of serial sections of spikelets selected from these inoculation experiments.

The greenhouse inoculations were incubated in controlled-temperature and humidity chambers at 12°, 20°, 24°, 28°, and 32° C. At 28° and 32°, the relative humidity was maintained at approximately 70 to 75 per cent; at lower temperatures it was decreased to obtain the same evaporation rate from the Livingston atmometer. During the incubation period in the field, humidity was increased by bagging the heads with parchment or glassine bags.

Demineralization with hydrofluoric acid made it possible to cut serial sections of entire spikelets.

Inoculation was most effective when heads were blossoming or had just passed the blossoming stage, though infection resulted from application of inoculum from the in-the-boot stage to the past-flowering stage.

The length of the incubation period varied with the stage of development of the head at the time of inoculation and with the temperature during the period of incubation.

When heads were inoculated before blossoming, the position on the head of the first spikelet to show infection and the progress of infection to other spikelets were suggestive of the place of beginning and progress of anthesis rather than of an independent advance through the rachis. It is thought that infection occurs at flowering time, or shortly after, rather than earlier, because of the presence of anthers or other degenerating tissues in the spikelets on which the fungus first develops as a saprophyte and from which it spreads to the interior of the flower, especially to the developing kernel. Infection was frequently observed to begin in anthers that had failed to clear the glumes. Retention of anthers was not dependent on infection, for counts of healthy and infected heads of Marquis wheat showed the percentage of caught anthers to be higher for the healthy than for the infected heads. The high percentage of caught anthers occurred in the spikelet range in which initial infections were found.

Neither macroscopic observations nor histological studies indicated that initial infection occurred on the outer surface of the glumes.

Clear cases of initial infection occurring on caught anthers were found in the serial sections. The path of the fungus through the various parts of the spikelet is indicated.

It is impossible to say how often infection originates in caught anthers, nor can it be said that infection never occurs except by contact with anthers or other degenerating tissues that serve as a base of operations for the fungus. This method, however, appeared to be the rule under the conditions of the experiments described.

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# OPHIOBOLUS ORYZINUS, THE CAUSE OF A RICE DISEASE IN ARKANSAS<sup>1</sup>

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## INTRODUCTION

In 1924, Melchers<sup>3</sup> quoted a report of an unidentified species of *Ophiobolus* on rice from Arkansas. In the fall of 1928, when rice-disease investigations in Arkansas were taken up by the writer, it was learned that the report had been made by H. R. Rosen, of the Department of Plant Pathology of the University of Arkansas, who had found the fungus on specimens of diseased rice sent in from the rice section of eastern Arkansas. It was found also that Rosen had made measurements of the asci and ascospores and had compared them with those of other graminicolous species. He had also secured cotype material of *Ophiobolus oryzinus* Sacc. from F. J. Seaver, of the New York Botanical Garden. The species of the Arkansas fungus had not been determined, however, and no inoculations had been made to establish its pathogenicity on rice plants.

Since 1928, this *Ophiobolus* has been found repeatedly on rice in the vicinity of Stuttgart, Ark.

The present paper reports the following steps in the investigation: (1) The description and identification of the fungus, (2) the description of the disease it produces, and (3) the results of inoculations on rice plants.

## REVIEW OF LITERATURE

Three species of *Ophiobolus* have been reported by Miyake (9),<sup>4</sup> Ito and Kuribayashi (6), and Saccardo (11) as parasitic on rice. In addition, one other species has been reported by Baker (1, p. 75-76) as causing a rot of rice straw. Baker states:

We have been making a good many observations on the rapidity of rotting of the rice stubble left in the fields. The common practice here, in harvesting, is to cut the panicles, leaving the bulk of the straw in the field, and this being difficult to handle with small native plows, is usually burned, on the ground. This is, of course, a destructive practice. Under favorable weather conditions the rotting of straw proceeds rapidly, and it soon reaches a condition which favors handling by small plows. In this process a number of very interesting fungi are concerned. \* \* \* Three others have been proven to be new to science, *Ophiobolus oryzinus* Sacc., \* \* \*.

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<sup>2</sup> Formerly assistant pathologist, Arkansas Agricultural Experiment Station. Acknowledgment is made to H. R. Rosen, of the Plant Pathology Department of the University of Arkansas, for cotype material of *Ophiobolus oryzinus*, which he had obtained from F. J. Seaver, of the New York Botanical Garden, and for specimens of the Arkansas fungus. The writer is also indebted to F. J. Seaver for additional specimens of *O. oryzinus*, and to Hurley Fellows, Division of Cereal Crops and Diseases for specimens of *O. graminis* on wheat.

<sup>3</sup> MELCHERS, L. E. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1924. U.S. Dept. Agr., Bur. Plant Indus. Plant Disease Survey Bul. Sup. 40: 107-188. 1924. [Mimeographed.]

<sup>4</sup> Reference is made by number (italic) to Literature Cited, p. 805.

Another species, called by Hara (5) *Ophiochaeta graminis* (Sacc.) Hara has been reported on rice. Hara identified this fungus as *Ophiobolus graminis* Sacc., but put it into the new genus *Ophiochaeta* because of the presence of bristly hairs on the beak of the perithecium.

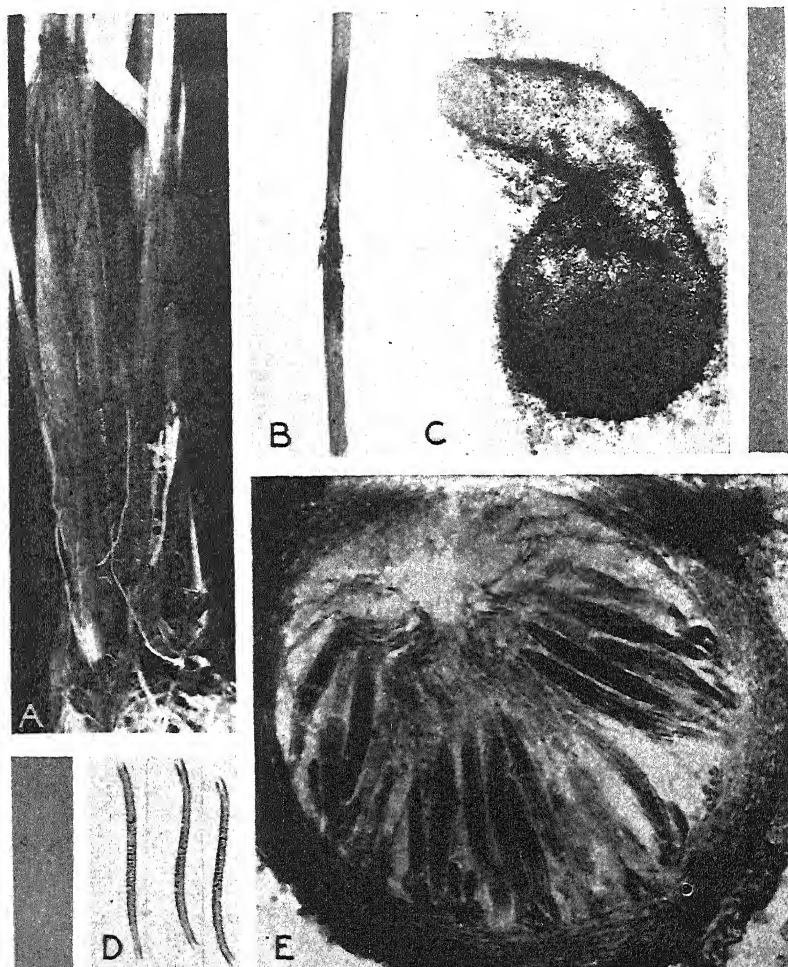


FIGURE 1.—A, Blue Rose rice plant infected with *Ophiobolus oryzinus* from inoculations made in the greenhouse at time of heading. Natural size. B, Seedling Blue Rose rice plant inoculated with *O. oryzinus* in the greenhouse.  $\times 3\frac{1}{2}$ . C, Perithecium of *O. oryzinus* partly embedded in the host tissue.  $\times 80$ . D, Ascospores of *O. oryzinus*.  $\times 300$ . E, Cross section of perithecium of *O. oryzinus*.  $\times 300$

## THE FUNGUS

### MORPHOLOGIC CHARACTERS

The Arkansas fungus may be described as follows:

Perithecia gregarious, globose to subglobose, embedded, grayish at first, becoming black to blackish brown at maturity,  $187\mu$  to  $375\mu$  in diameter; beak erumpent, hairy in culture only,  $125\mu$  to  $375\mu$  long; asci colorless, clavate or subcylindric, tapering at the ends with the apex slightly larger than the base, elongate, 8 spored,  $12.5\text{--}14.5\mu$  by  $96\text{--}111\mu$ ; paraphyses present, hyaline; spores fasciculate, long and slender, 3 to 5 septate, often multigutulate, usually crescent

shaped and often slightly spirally twisted, slightly constricted at the middle septum, apex rounded, base tapering,  $2-3\mu$  by  $79-112\mu$  (mostly  $86-111\mu$ ), buff to white in mass to the naked eye, but colorless when observed microscopically. On sheaths and culms of *Oryza sativa* L. (Fig. 1.)

#### IDENTIFICATION

A comparative study has been made of the morphologic characters of species of the genus *Ophiobolus* reported as causing diseases of rice, or species that might be present where rice is grown, in order to determine whether the fungus under consideration is identical with one of those previously described on *Oryza sativa*. The measurements, given in Table 1, show that several of the species differ distinctly from the fungus under investigation. In *Ophiobolus miyabeanus* Ito and Kuribayashi, *O. herpotrichus* (Fr.) Sacc., and *O. oryzae* Miyake the asci and spores are distinctly larger than in the fungus under study. However, in the other species studied, namely, *O. cariceti* (Berk. and Br.) Sacc., *O. graminis*, *O. oryzinus* Sacc., and *Ophiochaeta graminis* (Sacc.) Hara, either the asci or the spores have some characteristics similar to those of the fungus under discussion.

Comparisons were made with the specimens of *Ophiobolus graminis* on wheat obtained from Hurley Fellows and with specimens from the herbarium of the Department of Plant Pathology, University of Arkansas. Examination was made of cotype material of *O. oryzinus* collected by C. F. Baker and named by Saccardo (12). Two specimens of *O. oryzinus* have been examined, one from the New York Botanical Garden and one from the herbarium of the Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture. This latter specimen is No. 265, Fungi Malayana. Unfortunately, these two specimens are not identical. However, the specimen received from the Division of Mycology and Disease Survey agrees with the description given by Saccardo, while the other does not. The specimen from the New York Botanical Garden was compared with descriptions of various species of *Ophiobolus* known to occur on rice; it was found to agree most closely with *O. oryzae* (9). (Table 1.) A more detailed study, made on additional specimens, showed that this fungus was identical with specimens of *O. oryzae* found on Rexoro rice stubble in the vicinity of Crowley, La., during the winter of 1931-32.

After an examination of the available material and a study of the description of the species listed above, it was concluded that only *Ophiobolus oryzinus* agreed closely enough with the fungus under consideration to warrant further detailed comparison.

Further study of the cotype material of *Ophiobolus oryzinus* in the United States Department of Agriculture and a comparison of this material with the fungus obtained from rice in Arkansas proved that the two are at least morphologically identical. The wall structure of the perithecia was found to be the same, paraphyses were present in both specimens, and when mounted side by side the specimens were indistinguishable in color, size, and shape of asci as well as in septation, size, shape, and arrangement of the ascospores. The fungus under study was accordingly identified as *O. oryzinus*.

TABLE 1.—*Sizes of perithecia, asci, and ascospores of fungi as compared with those of the species of Ophiobolus found on rice in Arkansas*

Fungus	Investigators	Host	Size of—		
			Perithecia $\mu$	Asci $\mu$	Ascospores $\mu$
<i>Sphaeria curvati</i>	Berkeley and Broome (2)	<i>Ara caespitosa</i>	330-500 (average 425)	90-115 X 10-13	76.2-101.6,
<i>Ophiobolus caricis</i>	Ellingboek et al. (4)	Wheat, barley, rye, etc.			60-60 (chiefly 70-80) X 3.
Do.	Kirby (8)	Grasses and cereals		75-105 X 10.5-13.5	60-107 X 3-3.75.
Do.	Rosen and Elliott (10)	Wheat		80-100 X 10-13	60-60 X 3-3.5.
Do.	do.	Chenopodium		85-100 X 10-13	61-87 X 3-3.5.
Do.	do.	Reseda		80-80 X 12-13	61-88 X 3-3.5.
<i>O. graminis</i>	Saccardo (11, p. 349)	Cynodon	500-750		70-75 X 3.
Do.	Davis (3)	Wheat	300 (average)		70-80 X 3-5.
Do.	Jones (7)	Oats	240-480	80-120 X 8-16	72.1-101 X 3.2-4.
<i>Ophiobolus graminis</i>	Unger (6)	Rice	250	125-150 X 8-10	100-130 X 2-3.
<i>Ophiobolus oryzae</i>	Myake (9)	do.	500-950 X 308-777	142-235 X 21-30	250-408 X 6-9.
<i>O. nigrescens</i>	Uo and Kuribayashi (6)	do.	500-750	150-185 X 9-10	135-150 X 2-2.5.
<i>O. neopurpureus</i>	Saccardo (11, p. 352)	Grasses	300-350	95-110 X 7-11	86-100 X 2-3.
<i>O. purpureus</i>	Baker (1)	Rice	187.5-375	90, 25-111 X 12.5-14	79-112 X 2-3.
<i>O. sp.</i>	Tullis	do.			

\* Comparison made on cotype material.



## CULTURAL CHARACTERS

*Ophiobolus oryzinus* in culture grows readily on potato-maltose, potato-dextrose and corn-meal agar, and on other solid media. Abundant felty light-colored aerial mycelium is produced on potato-maltose and potato-dextrose agar, but on other solid media the growth is less abundant. On corn-meal agar only scanty aerial mycelium is produced. On nearly all media used, the submerged mycelium became dark in the course of several days. On liquid media the fungus grew poorly and did not fruit.

With corn-meal agar perithecia are produced in abundance, either on the surface or submerged in the medium. Most of the perithecia produced on agar have rather long beaks, although the beaks of some are short.

The use of single-spore cultures as detailed below indicates that the fungus is homothallic. Forty-one single-spore isolations were made, and 37 of the resulting cultures, incubated at room temperature, produced perithecia in two weeks. In these cultures mature ascospores were produced in 17 days. This indicates that the formation of perithecia does not depend on the presence of mycelium from more than a single ascospore. The exact length of time required for spore production on the host has not yet been ascertained, but it has been found to be considerably longer than 17 days.

If the perithecia are allowed to dry slightly, the ascospores are ejected forcibly. The ascospores germinate readily if taken just after they are discharged, before they have become dry. Germ tubes are produced from both polar cells but not from the intercalary cells.

## SYMPTOMS OF THE DISEASE

In several places in the rice-growing section of eastern Arkansas the disease has been observed in the field at various times since 1923, when it was first reported. Usually it is found rather late in the season, about the time when the water is drained from the fields preceding harvest. Some rotting of the sheath tissues occurs, however, before this time. Plants infected with *Ophiobolus oryzinus* are characterized by a brown discoloration of the sheaths from the crown of the plant to considerably above the water line. (Fig. 1, A, B.) In these discolored tissues the perithecia with their protruding beaks are found. The number of perithecia per plant may vary from 4 or 5 to several hundred. In the earlier stages of infection dark reddish-brown mycelial mats as much as 1.5 cm long and as wide as the sheath may be found on the inner surface of the diseased sheaths. As the lesions become older the mycelial mats increase in length, and when the sheaths become heavily infected the respective leaf blades die. At maturity the straw has a dull brownish cast very similar to that of plants attacked by the stem-rot fungus *Sclerotium oryzae* Catt. At times the two diseases have been found on the same plants or in the same fields, but parallel inoculations with the two fungi have shown that they are distinct.

Invasion of the rice culms proper by *Ophiobolus oryzinus* occurs, but perithecia are not commonly produced on them under field conditions. Under greenhouse conditions, however, perithecia have been produced on the culms.

*Ophiobolus oryzinus* injures the rice plant by killing the leaves and thus reducing the photosynthetic area. In some varieties, most noticeably in red rice, the tissues at the crown also may be killed. This is less evident in Blue Rose. Furthermore, the attacked mature plants grown in the greenhouse usually produce only a single head per plant and ripen prematurely. In the field also, tillering is reduced in infected plants, and frequently only one head per plant is produced.

## INOCULATION EXPERIMENTS

### PATHOGENICITY OF THE FUNGUS

To determine whether the fungus was pathogenic, the following artificial inoculations were made in the greenhouse on plants of Supreme Blue Rose, Fortuna, and red rice.

On January 1, 1929, five jars of Supreme Blue Rose rice plants were inoculated at time of heading with ascospores from pure cultures of the fungus. Of the 18 plants inoculated, 5 plants in four of the jars became infected.

On February 3, 1929, another series of inoculations was made, as in the preceding experiment, on nine Supreme Blue Rose rice plants at heading time. Five plants became infected.

On January 28, 1930, 73 Blue Rose rice plants grown in 15 jars seeded September 30, 1929, were inoculated with mycelium from a single spore isolation of *Ophiobolus oryzinus* made during the summer of 1929. Sixty-four of these plants became infected.

On January 30, 1930, three jars each of Blue Rose and Fortuna rice were planted. On February 10, 30 plants in each of two jars of each variety were inoculated with a spore suspension of *Ophiobolus oryzinus*. When the readings were made on May 27, 31.7 per cent of the Blue Rose rice plants and 25 per cent of the Fortuna rice plants were infected. All the control plants of Blue Rose were free from the disease, but three of the Fortuna controls were diseased. These were probably infected by water accidentally splashed into the jar from one of the inoculated lots of plants.

Eleven seedling plants grown on corn-meal agar in test tubes were inoculated with the organism on January 20, 1930. At the end of one month the plants were dead, whereas control plants remained green and healthy. Perithecia were produced during the latter half of March on the dead seedlings.

On March 13, 1930, three jars of seedlings of red rice were inoculated with a spore suspension of *Ophiobolus oryzinus*, immediately upon emergence. Three weeks later an examination of these plants was made and it was found that an average of 36.3 per cent of the plants were infected by the fungus.

The data given in the above text show that infection of Blue Rose and Fortuna rice plants, in both seedling and heading stages, was secured by artificial inoculation with ascospores of *Ophiobolus oryzinus*. Seedlings of red rice also became infected following similar inoculation.

### PATHOLOGIC HISTOLOGY

The invasion of the host is brought about by direct penetration of the outer epidermis of the two basal leaves or of the outer epidermis of the sheaths of older leaves. The two basal leaves may be invaded

directly by the hyphae. It appears, however, that appressoria are necessary for the invasion of older leaves or at least facilitate it.

Seedling rice plants were inoculated without wounding, by placing pieces of agar containing mycelium of the fungus in contact with healthy plants at the soil line. Leaves of plants so inoculated were removed and examined the second day following. The leaves were mordanted in 4 per cent iron alum, washed in water, stained for one minute in Heidenhain's haematoxylin, washed, cleared, and mounted in balsam for examination. Other material was fixed in chromo-acetic fixative, embedded, sectioned, and stained. Examination of all this material showed that the invasion was limited at first to the outer sheath, the mycelium soon penetrating to the space between the outer sheath and the one next under it. Here a mycelial mat was formed consisting of dark-colored rather coarse hyphae. Meanwhile branches of the invading hyphae penetrated the air chambers of the sheath and here balls of hyphae were produced, which developed into the perithecia. This invasion was accompanied by a slight darkening of the host tissue. Perithecia were produced for some time, and the lesion gradually increased in size until it involved the entire sheath for a distance of 4 to 6 cm from the original point of infection. Invasion of the underlying sheaths and the culm was facilitated by the production of appressoria. The tissues invaded died soon after discoloration occurred.

#### SUMMARY

*Ophiobolus oryzinus* Sacc., originally discovered by C. F. Baker on rotting rice straw in the Philippines, has been found to produce a disease of rice in Arkansas.

Inoculation experiments have shown that *Ophiobolus oryzinus* is pathogenic on Fortuna and Blue Rose rice plants in seedling and in heading stages, and on red rice plants in the seedling stage. Some of the plants have been killed outright and others have been injured through loss of leaf area. Red rice plants have been found also to have their crowns invaded. Infected plants failed to tiller normally, producing tillers only after the heading of the first culm.

Invasion of the host occurs by direct mycelial penetration of the epidermis of the basal leaves. Appressoria are formed and aid in the invasion of tissues under the basal leaves.

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# SCLEROTIA-FORMING HABITS OF THE COTTON ROOT-ROT FUNGUS IN TEXAS BLACK-LAND SOILS<sup>1</sup>

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## INTRODUCTION

The cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dug., apparently has little difficulty in living through the winters in most districts. The disease has not, however, been observed north of the southern tier of counties in Oklahoma; its present northern limit coincides approximately with the location of the Red Valley.<sup>2</sup> In individual areas, infection may fail to appear in successive years except in a few centers representing only a very small fraction of the original area involved. This, however, applies only to individual areas of infection and is independent of the general or regional behavior of infection.

Experiments with clean fallows have thrown much light on the manner in which the fungus perpetuates itself in the soil and lives through the years to attack the next susceptible crop planted. Plots kept in clean fallow for three or four years and then returned to cotton show a material loss in plants, indicating definitely that the fungus may remain in the soil in a viable condition for a period of several years without the presence of living host material. It is now known that sclerotia play an important part in carrying infection over into succeeding seasons.

The present paper reports the results of careful examination of the soil surrounding the first plants to die on plots that had been returned to cotton after having been in clean fallow or planted to nonsusceptible crops for several years. From extensive field examinations made during the summer and fall of 1931, it appears that the prolonged persistence of the disease in clean fallows and in plots planted to nonsusceptible crops is undoubtedly due to sclerotia formed below the plow slice and associated with various sorts of dead and decaying material such as tree stumps or old roots.

## HISTORICAL REVIEW

Some of the earliest investigators (1, 7)<sup>3</sup> of cotton root rot advocated aeration of the soil as a means of control. This remedy was suggested on the assumption that the root-rot organism is a parasitic fungus living and reproducing entirely by vegetative growth. Since then, however, much additional work has been done on the fungus in culture, and many kinds of control methods have been tried in the field.

<sup>1</sup> Received for publication Oct. 29, 1932; issued June, 1933.

<sup>2</sup> Information furnished by F. M. Rolfs, of the Oklahoma Agricultural and Mechanical College.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 819.

None of the field experiments has given consistently favorable results, which indicates that more subtle factors are involved than had been recognized.

On the assumption that the causal organism is an obligate parasite, control experiments with clean fallows were begun in 1922 and have continued since that time (3). Although the early results appeared promising, the short-time fallows were found to be unsatisfactory in many respects. The longer the period of clean fallow, as a rule, the greater appeared to be the control of the disease when the plot was returned to cotton. Taubenhaus and Killough (8) in 1923 recommended clean culture and rotation with clean culture, combined with the destruction of all winter carriers, as a means of control. In general, the results with clean culture alone have not been satisfactory, and frequently plots cropped continuously to cotton may, when the infested area breaks up, show as little loss from the disease or even less than plots that had been in clean fallow two or three years (4).

In 1929, Ratliffe (6) described a prolonged saprophytic stage of the organism, and King and Loomis (2) announced the discovery of a sclerotium stage of the fungus in laboratory cultures in Arizona. Later in the same year the occurrence of sclerotia at Greenville and San Antonio, Tex., was reported by Neal (5). With these discoveries the whole line of attack upon the problem was changed. The discovery of the sclerotium stage explained many of the inconsistencies in the behavior of clean fallow and rotation experiments. These discoveries also advanced field investigation to a stage where it was clear that the problem involved more than the control of a strictly parasitic fungus.

#### METHODS OF FINDING SCLEROTIA IN BLACK-LAND SOIL

The soil of the United States Cotton Breeding Field Station near Greenville, Tex., is composed largely of Wilson clay, which, even under very dry conditions, is easily broken apart in the hands, exposing the rootlets and fungus growths of current or former seasons. These growths find ready access through the soil along the well-formed cleavage planes, which are very well marked and are probably the result of years or centuries of continued movement of relatively small soil masses. Probably because the soil alternately expands when moist and contracts when dry, the effect of these irregular cleavage planes is to allow it to be readily broken apart. The surface of the cleavage planes from the lower depths is usually well coated with colloidal matter. Sometimes they have the appearance of small slickensides. These physical characteristics are very similar in the Wilson and Houston clays. (Fig. 1.)

For the first 2 to 4 inches the surface soil is loose and granular. Below this layer of loose soil is a very tight, close-grained stratum which might be termed the plowsole. Whether or not this truly represents a plowsole is a question, but such a condition was present in all Wilson and Houston clays examined. This close-grained layer of soil is usually stratified horizontally, and it is of course dissected vertically by cracks. Occasionally the fracture is conchoidal. This layer is difficult for the plant roots to penetrate, and many of the rootlets are found along the horizontal cleavage planes which separate one close-grained stratum from another. In this zone there is much less evidence of an accumulation of colloidal material along the cleavage lines than exists at lower depths.

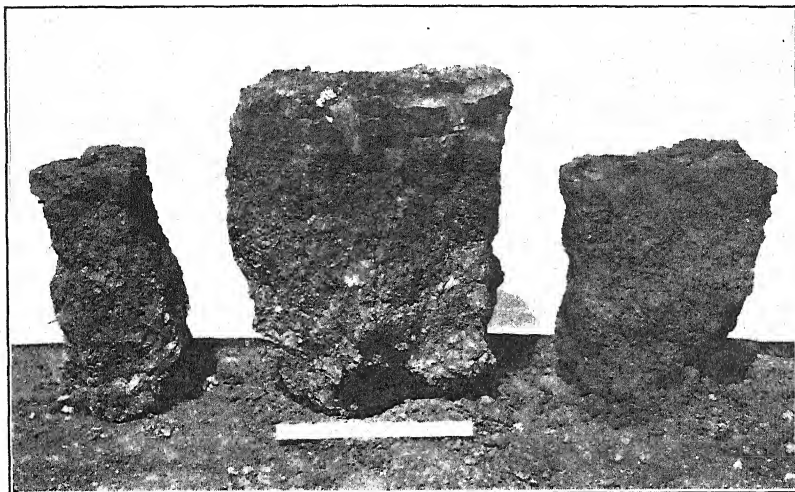


FIGURE 1.—Typical profile of Wilson clay. Note fine-grained stratum near top of the blocks. Sclerotia occur in greatest abundance below the fine-grained stratum, which is not penetrated in ordinary tillage operations. Root channels from plants grown at least five years previously are shown on the right. The loose topsoil has been removed



FIGURE 2.—Large clod of Houston black clay soil, outlined in black, showing natural position of viable sclerotia and cleavage planes. (Two-thirds natural size.) Photographed by P. R. Dawson. Austin, Tex., November 7, 1931

Below the plowsole, even during the driest part of the summer, the larger "chunks" can be easily broken apart with the hands and examined in the field for root-rot infection, sclerotia, and root development. Figure 1 shows the close-grained nature of the plowsole as compared with the more friable subsoil, which may frequently change abruptly in color and composition. Old root channels from a cotton crop grown five years previously are also to be seen in this figure. In these examinations sclerotia were found only in soil with a physical structure that would readily allow the penetration of rootlets and root-rot mycelium. In practically no case were sclerotia found inside a piece of compact soil so hard and tough that it could not be broken apart in the hands.

Sclerotia are readily visible in both the Wilson and the Houston black clay soils. (Fig. 2.) In soils of a lighter color and with a higher content of sand, field examinations for sclerotia are much more difficult.

The simple method of breaking the soil apart in the hands and exposing the fungus growth and the sclerotia has been found much more satisfactory than washing them out of heavy clay soils like the Houston and Wilson. This method also has the advantage of being rapid and of requiring the handling of a minimum amount of soil. It also affords a readier observation of the sclerotia in situ and makes it possible to obtain more definite information on the habits of the fungus.

#### OCCURRENCE OF SCLEROTIA BELOW PLOW DEPTH

The examinations were begun on July 16, 1931, and continued at short intervals for the remainder of the year. Observations were made throughout the summer and fall as often as possible, sometimes daily, but at least once every two weeks. The detailed examinations involved the handling of many tons of earth and revealed the fact that sclerotia generally are formed in groups or colonies, the great majority of which occur below the plow slice at depths usually ranging from 6 to 12 inches below the surface. A reason for the occurrence of sclerotia in greater abundance within the surface foot of soil at Greenville is that the plant roots are very sparse at lower depths. Sclerotia are evidently laid down in the soil near the small rootlets and not on the taproot or larger laterals. They are found along the old channels of the rootlets or near the fungus strands which had destroyed the small rootlets. In several cases the sclerotia were laid down in the old root channels of the 1926 cotton crop. Strands of the fungus are usually found with each colony of sclerotia. Unfortunately there is as yet no way of determining the age of sclerotia in the soil, although there is much evidence to indicate that they may be sealed in the clay and remain in a viable condition for long periods, probably several years.

#### INVESTIGATIONS AT GREENVILLE

##### SCLEROTIA IN PLOT D-1

In 1931 the west half of plot D-1 was planted to cotton, after four years of clean fallow (from 1927 to 1930), and the east half of the plot was clean fallowed for the fifth consecutive year.



Several neighboring plants died on this plot between July 20 and August 6, when the first examination was made. Diggings around the first plant that died did not yield sclerotia, but showed numerous heavy strands of hypha. Near an adjacent plant, which had been wilted only a few days, sclerotia and hyphal strands were found in abundance. Both freshly formed white sclerotia and older dark-brown ones were present. Masses of sclerotial casts were also found, indicating that sclerotia had been formed some time previously, possibly several years before, and that the growth from these old casts may have been the source of the infection present in this particular location. Most of these sclerotia were laid down near the taproot and laterals at depths of 6 to 8 inches below the surface. At 11 to 12 inches the subsoil changed from a heavy black clay to a yellowish gray clay with numerous calcareous concretions.

The third plant to die was growing in a very tenacious black clay. The surface moisture was abundant, and many shallow lateral rootlets had developed near the surface. Here, both strands and sclerotia were found within 2 inches of the top of the soil. The taproot was infected to a depth of about 10 inches, but was sound below. On an adjacent plant sclerotia were not found near the surface but at a depth of 6 to 8 inches.

A further examination of this area of infection was made on August 17. Between the first two rows next to the 5-year fallow a large colony of sclerotia was found, all of which were old and very dark brown in color. About 12 inches from a dead plant in the outside row a second colony was found which showed a large number of light-colored sclerotia, evidently very recently formed. Older sclerotia of course were found in this location also, and numerous hyphal strands in both colonies.

Another primary center of infection on this plot appeared during the last week in August. This center was represented by two dead plants. On September 28 the soil around both plants was examined and two colonies of large sclerotia were found. The first group of sclerotia was uncovered at a depth of about 5 inches below the surface and about 9 inches from the first dead plant. The second colony was found at about the same depth and about 10 inches from the second dead plant. The sclerotia in one of these groups were the largest found during the season. All appeared to be very old, but some were viable.

Another examination was made in this area on October 1, and large numbers of sclerotia and sclerotial casts were found. Most of them were found about 18 inches from one of the dead plants and at a depth of 4 to 6 inches. Since no cotton was grown on this plot in 1930, the casts probably represent sclerotia that were laid down in 1926 or 1927 and that were doubtless the primary source of infection for this center.

On September 2 a third primary center of infection was observed on the west half of plot D-1. There were seven dead plants in one row, and an examination of the soil revealed a large amount of rubbish such as bits of decayed wood, peach seeds, nails, glass, brickbats, charred wood, etc. A large colony of sclerotia was found about 10 inches from the first plant that died. Two days of continued digging failed to disclose any other source of infection, although the root-rot

map of the area for 1920 shows trees and grass within 10 feet of the location of the first dead plant.

Another primary center of infection in the same row as the one previously described was examined on November 12. This center consisted of three plants which died very late in the season. The plowsole was very well marked here, and at a depth of about 7 inches numerous old sclerotial casts and old sclerotia were found. Old peach seeds and well-rotted bits of cotton stalks or roots were found near the dead plants.

Aside from the presence or absence of dead tree roots, the sclerotia uncovered appeared to be very old, as indicated by their color, condition, and location in old root channels. The casts appeared to be the remains of sclerotia that had also been laid down several years previously. Some of the oldest looking sclerotia, which had been formed near root channels that were undoubtedly from the cotton crop of 1926, when placed in moist chambers put out new growth within two days.

Another center of infection in the same row was examined on November 13. A few old sclerotial casts were found, together with considerable organic matter which appeared to be the remains of a former rubbish pile, but no true sclerotia were discovered. The examination was resumed on November 16, and numerous sclerotia of considerable age were found. Although there is no method of determining the exact age of sclerotia laid down in the soil, indications of their having been freshly formed are found in their color and in the condition of the root material surrounding them. The fact that plants were dying in the same row at three different points and that peach seeds occurred at each of these points indicates that at some previous period there was a row of peach trees across the plot.

Since the largest body of infection in the cotton on the west half of plot D-1 was contiguous to a 5-year clean-fallow plot, an examination for root-rot infection was made in the fallow. As there were no plants to indicate the possible locations of infection in the soil, the examination was very tedious and lasted from August 24 to 28. Most of the work was done at a distance of 10 feet or more from the first row of cotton in the adjacent plot. No sclerotia were found at these distances, although one colony was discovered about 18 inches and another about 3 feet from a dead plant in the outside row of cotton next to the clean fallow. Sclerotial casts were found at a distance of 6 feet from the cotton. The decomposed remains of what appeared to be the root system of a shrub were uncovered at depths of 4 to 5 inches. This examination was rather thorough, for the area examined, but by no means conclusive. The simplest and most reliable method of testing the plot for infection will be to plant it in cotton. As in the case of the 4-year clean fallow, the presence of dead plants will show that the fungus has maintained itself for a period of practically five years without live host material.

#### SCLEROTIA IN PLOT D-2

Plot D-2 had been in clean fallow during the years 1927, 1928, and 1929. When the plot was planted in cotton in 1930, dead plants appeared at two points centrally located on it. The first plant that died on the plot in 1931, however, was near a corner where there had

been no mortality in 1930. On July 16, when the soil near this dead plant was removed, two large cotton stumps from the outside row of the 1930 crop were uncovered and were found to be supporting a heavy growth of the root-rot fungus. (Fig. 3.) There was no doubt of the contact between the live roots of the 1931 planting and the old roots of the 1930 planting. No sclerotia were found on July 16. A small amount of soil in this location was again examined on August 6, but no sclerotia were found. On August 17 two plants died, one next to the first dead plant and the other in the adjacent row on the inside of the plot.

On August 25 digging was resumed and two colonies of sclerotia were found about 5 feet from the first plant that died, one at a depth of 5 to 6 inches and the other at a depth of 14 to 18 inches, directly under the first. Some of the sclerotia appeared to be very old and

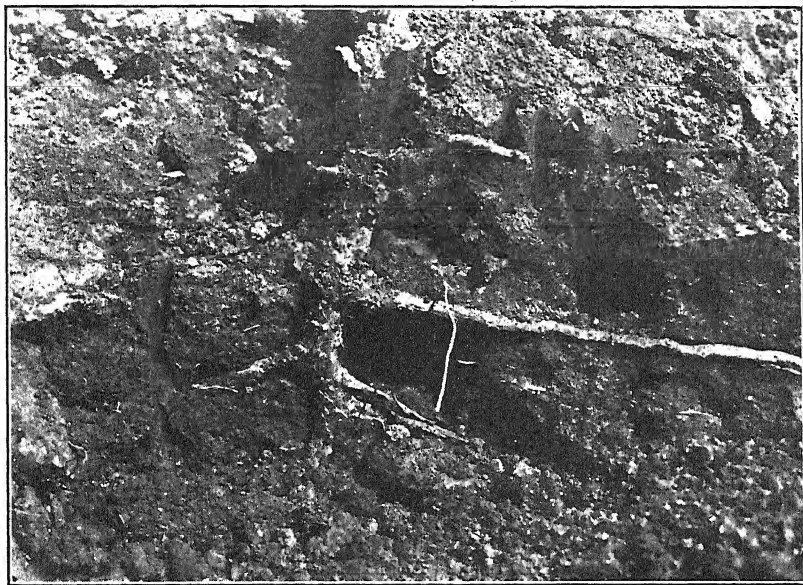


FIGURE 3.—Cotton stump from previous season's planting near primary center of infection, supporting a heavy growth of the root-rot fungus. Greenville, Tex., July 16, 1931

evidently had been formed before August 6, although none was found in the soil examined on that date. In one case there was evidence of the older sclerotia producing new ones by budding, as described by King and Loomis (2). Just below the plow depth an old peach-tree stump was found almost directly in contact with the first dead plant in the second row. This tree had been dead at least 13 years. (Fig. 4.) Large numbers of sclerotia were found near this stump, at distances of 12 to 18 inches. A point of interest in connection with this particular location is that while numerous sclerotia and sclerotial casts were found, none of them was in direct contact with either live or dead roots, but all were near dead roots and near the dead peach-tree stump. All sclerotia found were below the plow depth. Although no plants had died in 1930, when this area was planted in cotton after a 3-year clean fallow, much significance may be attached

to the fact that the first plants to die in 1931 were near the dead peach-tree stump, which was surrounded by several colonies of sclerotia, some of which appeared to have been in the soil for a long time.

The soil around a second primary center of infection on this plot was examined on July 16. The first plant had died on July 12. Although no dead material other than cotton stalks and roots was observed, large numbers of sclerotia were found, some of them evidently several weeks old. An interesting feature in connection with the examination of this center was the exposure of a shallow area in which the subsoil came within about 3 inches of the surface. The subsoil was mottled and yellowish gray in color, containing numerous small calcareous concretions. The surface soil of the Greenville station is classed as Wilson clay and is not calcareous.



FIGURE 4.—Old peach stump uncovered below plow slice and near primary center of infection. Sclerotia were found in profusion near this stump. A history of the plot indicates that this tree has been dead for not less than 13 years. Greenville, Tex., August 25, 1931

#### SCLEROTIA NOT FOUND IN CONTINUOUS COTTON PLOTS SHOWING VIRULENT ROOT-ROT INFECTION

Since sclerotia had been found near all primary centers of infection on plots D-1 and D-2, which had been in fallow four and three years, respectively, it was decided to make examinations in other parts of the station.

The location selected for this study was in a field which had been continuously in cotton for several years and in which nearly all the plants had died. The area was about 900 feet distant from plots D-1 and D-2. The soil was carefully worked from around the dead plants near the central part of the infested area to several feet beyond its outer limit in the area of healthy green plants.

Trenches about 20 to 26 inches deep were dug between two rows and the soil was carefully worked down from around both the dead plants and the green plants on the outer edge of the area of infection. The trenches were usually 18 to 20 feet long, and some of them were near the center of the infested area. Examinations of this area were begun September 3 and were continued at frequent intervals until November 13; during this time many tons of earth were handled and not a single sclerotium was found. No casts of sclerotia that might have been laid down at some previous time were discovered. Several cursory examinations were made in near-by areas of infection on similar types of soil having the same crop history, but no sclerotia were found. Why this area with a heavy, early mortality of plants should have failed to yield sclerotia, when they were being found in abundance on the plots that had been in clean fallow for a period of years, is a question of genuine interest. Both shallow and deep phase soil was encountered in a number of places within the infested area, although apparently it exerted no favorable influence upon the formation of sclerotia. It is also of interest to note that repeated examinations along the outer margin of infection failed to yield sclerotia. On October 1 an examination of several primary centers of infection on plot C-6 was made, but no sclerotia or sclerotial casts were found. This plot had been in cotton continuously for at least 13 years and probably much longer.

#### INVESTIGATIONS IN TRAVIS COUNTY

In 1927 an experiment was begun on the farm of W. F. Voelker near Manor, Travis Co., Tex., for the purpose of determining the effectiveness as a control measure of a 3-year clean fallow as compared with that of three years of nonsusceptible crops on land very heavily infested with root rot. Two plots of approximately 1 acre each were laid off. One plot was kept in clean fallow during a 3-year period, while the other was planted to oats in 1928, sorghum in 1929, and corn in 1930.

When the plots were visited on June 23, 1931, numerous dead plants were found, probably three times as many on the clean-fallow side as on the cultivated side. When observed again, August 12, fully one-half of the plants on the clean-fallow side were dead. Upon inquiry it was found that practically all the plot that had been in clean fallow for 3 years represented the site of an old peach orchard that had died out about 10 years before. Mr. Voelker states that in 1918, when most of the orchard had died out, some of the trees were replaced with young ones that lived only a few years and died out with the older trees. This information led to the belief that if a number of the primary centers of infection were investigated the source of infection might be determined. Accordingly, on November 5 and 6 several of the primary centers were examined.

The first center of infection examined yielded a number of peach seeds, and an old dead mesquite stump about 12 inches long and about 1¼ inches in diameter. Sclerotia and sclerotial casts were found in abundance about 5 inches below the surface. (Fig. 5.)

An excavation about 6 feet square, made near the second center of infection, revealed the location of an old rubbish heap containing peach seeds, pieces of board, cans, an old broom, bones, and finally

a well-rotted peach stump. The root-rot fungus was represented by numerous large strands, but no true sclerotia were found.

The third center of infection examined revealed an old tree root, which was less than half an inch in diameter but which, when traced

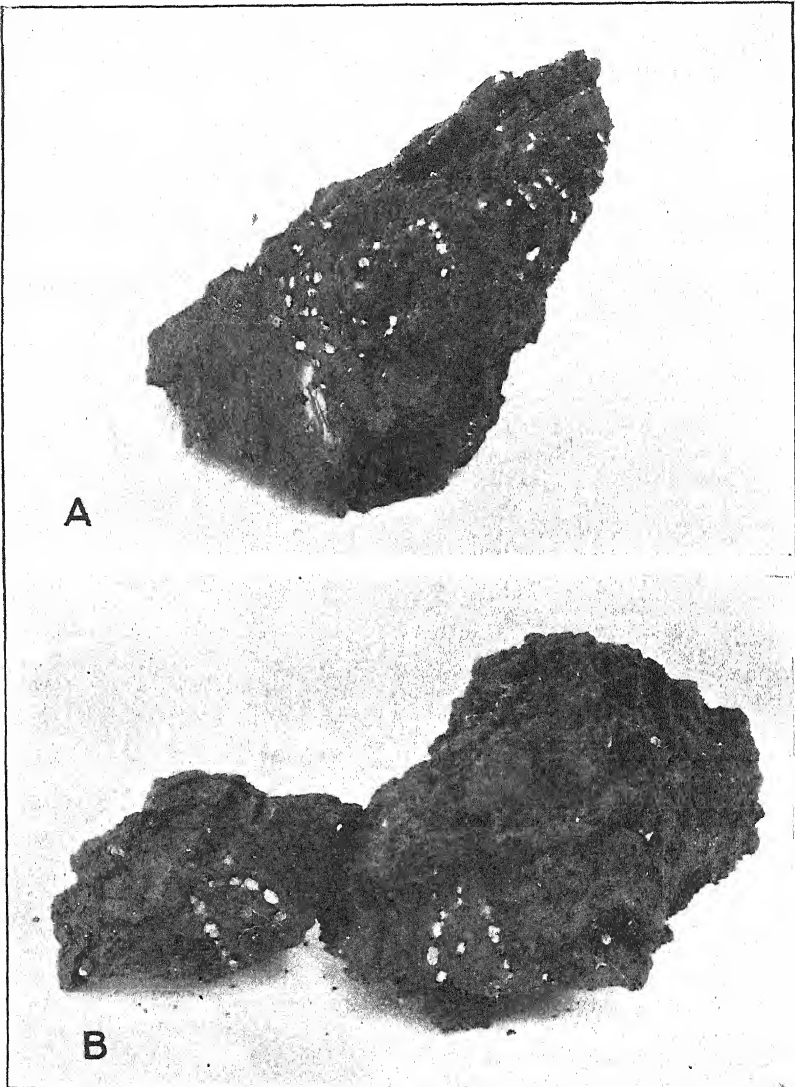


FIGURE 5.—A, Sclerotial casts as they occur naturally in chainlike formations in the soil. B, Smaller portion of clod broken away from larger portion, splitting the sclerotial casts bilaterally. (Four-fifths natural size.) Photographed by P. R. Dawson. Austin, Tex., November 7, 1931

out, was found to extend across about eight rows of cotton. Apparently the stump had been taken out and only the lateral roots left in the soil. The roots were very well rotted and numerous sclerotia and sclerotial casts were found in close proximity.

The fourth center of infection revealed the presence of an old tree root below the plowsole, which was found to connect with the old stump in the second center examined, as described above. No sclerotia were found in this center of infection.

The fifth center of infection yielded abundant sclerotia and sclerotial casts at depths of 10 to 12 inches.

Three other examinations were made in the 3-year-fallow plot, two of which yielded a number of peach seeds but no sclerotia, while one gave neither sclerotia nor other evidence of the source of the infection. These later examinations, however, were not so thorough as they might have been, and possibly the seat of infection in these points was overlooked.

Negative evidence in cases of this kind is not entirely reliable. The source of infection might be small and in many cases might have been destroyed or even overlooked unless much time were spent in the tedious process of examining the field.

A point of unusual interest was revealed in the examination of a well-defined primary center of infection on the plot that had been planted to nonsusceptible crops for three years. In examining this center, sclerotia and sclerotial casts were found in great abundance. Near one side of the area of infestation two small mesquite stumps were discovered which had been dead for years and around which was a great number of sclerotia. A rather sparse growth of mycelium extended over the mesquite stumps, and sclerotia were present 2 to 4 inches from the stumps and 6 to 15 inches below the surface. According to the owner of the land, the plot had been put into cultivation in the spring of 1884 and had had a crop planted on it every year since.

Recurrence of root rot after several seasons of clean fallows or of nonsusceptible crops is explained by deep-seated infestation of the fungus below the plowsole. The fungus was found in many cases on decayed roots of trees buried in the soil for long periods, in some instances for 30 or 40 years.

#### INVESTIGATIONS IN ROCKWALL COUNTY

On November 19 an examination of the soil was made around several primary centers of infection on the farm of B. F. Weatherford, near Rockwall, Rockwall Co., Tex. The soil would probably be classed as an eroded phase of Houston clay. The loss from root rot on this type of soil is extremely high.

The field examined had been planted to oats in 1928 and 1929, to millet in 1930, and to cotton in 1931. Each summer the plot was kept in fallow, and practically no weeds were allowed to grow on the area.

On July 27 there was a scattering of dead plants over the plot, some of which had died a few weeks earlier. After the mapping on November 19, an examination was made of five primary centers of infection. Four of the centers yielded sclerotia and sclerotial casts in abundance, but in one of the locations no sclerotia were found. Two explorations were made in an area of general infestation, but no sclerotia were found.



## SUMMARY AND CONCLUSIONS

In the study of the sclerotia-forming habits of the root-rot fungus (*Phymatotrichum omnivorum* (Shear) Dug.) reported herein, a simple method of investigation was employed in which the soil was excavated from different depths and broken apart in the hands. Thus the sclerotia were observed in situ, and their relation to small root channels and cleavage planes of the soil was readily seen. It was difficult to wash the sclerotia out of the black clay soil, but by breaking the clay apart it was easier to find them than in the more friable soils.

To determine the seats of infection the soil was carefully excavated and examined to depths of at least 24 inches around numerous primary centers where the first plants died of root rot. These examinations were made throughout the summer and fall of 1931, in fields planted continuously to cotton, in others planted in cotton for the first time after 3-year or 4-year clean fallows, and in still others where cotton followed three years of nonsusceptible crops.

Sclerotia were frequently found in the soil, usually in colonies or groups rather than singly or sparingly.

After the seat of infection in a primary center had been located, continued digging failed to reveal other sources. Although tons of additional earth were removed, no more sclerotia were found.

Older sclerotia were found below the plow slice at depths of 4 to 18 inches, most of them occurring at a depth of 4 to 12 inches.

Sclerotia were not found on the taproots of the cotton plants or on the large lateral roots but out in the soil where only small rootlets occurred.

Newly formed sclerotia were found only during the period from August 6 to 17.

No sclerotia were found in plots planted continuously to cotton where most of the plants had died of root rot. They were, however, found in abundance in primary centers of infection in fields where cotton followed 3 or 4 years of clean fallow or 3 years of nonsusceptible crops.

Sclerotia were frequently found in large numbers near old tree roots or stumps that had been dead many years. Some of the primary centers of infection, however, although near old dead roots, failed to yield sclerotia.

Most of the primary centers, after three years of nonsusceptible crops or 3-year fallows, yielded either sclerotia or infested old decayed material of some kind, or both.

Where deep-seated infestations exist, control of root rot by clean fallows or nonsusceptible crops apparently will need to be supplemented by tillage operations that will reach the sclerotia, which are usually found below the plow slice at depths of 4 to 12 inches.

Considerable significance may be attached to the fact that in most of the primary centers examined sclerotia were abundant in plots that had been planted to nonsusceptible crops or kept in clean fallow for several years, whereas no sclerotia were found in plots planted continuously to cotton. From these facts it may be inferred that the sclerotia, which apparently are capable of long periods of quiescence, are connected primarily with the saprophytic phase of the fungus and serve as a resting stage.



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# ARTIFICIAL TRANSMISSION OF SUGARCANE MOSAIC<sup>1</sup>

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## INTRODUCTION

Sugarcane mosaic is a disease caused by a conceivable but as yet undistinguishable virus, which has been recognized during the last decade as affecting sugarcane (*Saccharum officinarum* L.) to some extent in almost every region where sugarcane is grown commercially. For a long time investigators failed in their efforts to infect plants experimentally with the virus of this mosaic disease, and the question arose as to whether it should be considered infectious or whether the symptoms observed in sugarcane might be caused by inherent degeneration or by a nonparasitic disease. The truly infectious nature of sugarcane mosaic was firmly established about 10 years ago, but many problems still remain unsolved.

Concerning the character of the virus in the extracted plant juice, the general belief has been that if manipulated in the presence of air it would wholly, or at least largely, lose its infectiousness, particularly if inoculations were not made immediately after extraction. This belief, which was not thoroughly tested, became a serious handicap in the study of the virus. This paper presents a procedure developed during the past year for the artificial transmission of the mosaic disease, which offers not only considerable freedom in the manipulation of the extracted material but also permits the use of the virus-containing extract for a considerable time after storage. The results here recorded show that exposure to the air does not necessarily impair, under all conditions, the disease-producing properties of the extract of diseased tissues. The studies were begun in the autumn of 1929 at Johns Hopkins University and have been continued in the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture.

## HISTORICAL REVIEW

The disease now known as sugarcane mosaic has been the subject of investigations and reports by various workers, first in Java (31),<sup>3</sup> later in Hawaii (25), and more recently in the West Indies (11, 16, 30, 33, 34, 35, 36, 37), the United States of America (6), Argentina (17), Natal (27), and India (29). In 1892 Van Musschenbroek (31) gave a description of the disease under the symptomatic name "gele strepenziekte," or yellow-strike disease, and in 1893 there appeared a multicolor reproduction showing a portion of a sugarcane leaf affected

<sup>1</sup> Received for publication Dec. 19, 1932; issued June, 1933. Data herein presented were included in a thesis submitted to the faculty of the Graduate School of the Johns Hopkins University, June, 1932, in partial fulfillment of the requirements for the degree of doctor of philosophy.

<sup>2</sup> Acknowledgment is made to Dr. E. W. Brandes, principal pathologist in charge, Division of Sugar Plant Investigations, for his contribution of many observations and much constructive criticism, and to Prof. Burton E. Livingston, of the Johns Hopkins University, for his help in the presentation of the results.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 837.

with the disease, together with a note on its occurrence, by Arendsen Hein (3). Since 1919 the disease has been recorded (6, 7, 8, 21, 39) as affecting not only sugarcane and other species of *Saccharum* but also maize (*Zea mays* L.); sorgo (*Sorghum vulgare* Pers.) and other species of *Sorghum*; millet (*Pennisetum glaucum* (L.) R. Br.); and the wild grasses *Digitaria sanguinalis* (L.) Scop., *Paspalum boscianum* Flügge, *Setaria lutescens* (Weigel) Hubb., *S. magna* Griseb., *Panicum dichotomiflorum* Michx., *Eleusine indica* (L.) Gaertn., *Echinochloa crusgalli* (L.) Beauv., *E. colonum* (L.) Link, and *Brachiaria extensa* Chase.

In 1903 Kamerling (19), working in Java, stated that he had transmitted the disease to healthy sugarcane by inoculation with juice expressed from mosaic-diseased sugarcane. His inoculations were made by injection. Although he did not specify the kind of instrument used, it was presumably a hollow-needle syringe, since he stated that he had followed the technic employed by Beijerinck (4) in transmitting tobacco mosaic to healthy tobacco plants, and Beijerinck stated that he used a hollow-needle syringe ("Pravaz'schen Spritze"). Kamerling, regarding this sugarcane disease as infectious, placed it in the same class with the well-known mosaic disease of tobacco. His tests, however, do not appear to have fully justified such an unqualified conclusion, for some of his uninoculated and apparently healthy plants contracted the disease in the course of his experimentation. He admitted the possibility that the disease might have been transmitted in a very slight degree by air, but he expressed the conviction that at least some of the plants which he inoculated with diseased juice contracted the disease directly because of his inoculation. On the other hand, Van der Stok (38) in 1907, Kobus (20) in 1908, and Wilbrink and Ledebøer (42) in 1910—all of whom, like Kamerling, studied the disease in Java—were unable to infect healthy sugarcane plants by any of the methods they tried, including the procedure described by Kamerling.

In 1917 Stevenson (35, 36) reported the results of a study of sugarcane mosaic in Puerto Rico, referring to it as the "new disease," "the mottling disease," "an epiphytotic of cane disease," etc. It was not until 1919, however, that he identified the disease as it occurred in Puerto Rico with the gele strepenziekte, or yellow-stripe disease, described by others. Working thus independently he suspected the disease to be due to an infectious principle (37) and carried out an extensive series of inoculation tests, not only employing expressed juice from diseased cane but also inserting diseased cane tissue in incisions made in healthy plants; but he did not succeed even once in causing the disease symptoms to appear in the inoculated plants.

Stevenson's paper of 1917 (35) aroused the interest of other investigators, particularly H. L. Lyon (as reported by Colón (14)), of the Hawaiian Sugar Planters' Association, who regarded this disease as identical with the disease known as gele strepenziekte, or yellow-stripe disease, in Java, Hawaii, and elsewhere. The yellow-stripe disease had been studied by Lyon in Hawaii from 1911 to 1914 (25). In 1921 (26) he reported that in open-field experiments he had observed it in some of the plants which he had inoculated with expressed juice. Similar tests in the greenhouse failed, however, to produce the disease.

The identity of the disease causing mottling of sugarcane in Puerto Rico with the gele strepenziekte of Java and the yellow-stripe disease

of Hawaii and other regions was finally recognized by the investigators of this subject in Puerto Rico (6, 14, 16, 30, 37), and the infectious nature of the disease was generally accepted by them. Adequate evidence of its being infectious was still lacking, however; the experimental evidence was meager and not free from the possibility of error; and nothing was known regarding field dissemination.

In 1920 Brandes (7) published the outstanding discovery that sugarcane mosaic is transmitted naturally by *Aphis maidis* Fitch. Additional evidence showing the infectious nature of this mosaic and the rôle played by *Aphis maidis* in its dissemination was presented by Bruner (11) in Cuba, by Kunkel (22) in Hawaii, by Ledebøer (24) and Wilbrink (40) in Java, by Chardon and Veve (12, 13) in Puerto Rico, and by Fawcett (17) in Argentina.

Early in 1920, Brandes (7) succeeded in transmitting the mosaic disease directly from diseased to healthy sugarcane without the aid of *Aphis maidis*. By means of a glass hollow-needle syringe, he injected into the growing points of healthy sugarcane plants 0.5 cc of sap pressed from ground tissue of young joints of mosaic-diseased sugarcane. After an interval of a little more than one month, infection had appeared in 8 out of 10 plants inoculated with sap expressed under a cover of purified mineral oil, but only 2 out of 10 inoculated plants contracted the disease when the sap employed was obtained without any protective covering against exposure to air. It is important to note, however, that in both instances the inoculating material was used, as the author states, "immediately after being prepared."<sup>4</sup>

Brandes and Klaphaak (10) regarded lapse of time and exposure to air as the chief causes of the failure of expressed virus-containing juice to produce the disease. They say (10, p. 251)—

Virus capable of causing infection when used immediately after being expressed from diseased stalks was found in one experiment to be without effect when injected 24 hours later.<sup>5</sup> The virus of grass mosaic is less stable or more sensitive to the influence of its environment than that of many other similar diseases, notably the tobacco mosaic. In these experiments it has been found very refractory and difficult of physical manipulation or chemical treatment without loss of virulence.

They also remark (10, p. 248): "Sometimes whole series of inoculations including known susceptible control plants failed, owing to unknown causes." These authors continued to use a cover of mineral oil over their inoculum, and they succeeded in transmitting the disease directly from cane to cane and from cane to sorgho, millet, and *Digitaria sanguinalis*. Thus it was definitely established not only that sugarcane mosaic is really infectious and transmissible to healthy plants of sugarcane and some related forms by the vector *Aphis maidis*, but also that it may be artificially transmitted by means of hollow-needle inoculation with freshly extracted sap from diseased plants suitably protected from the air.

Doolittle (15) found that juices of mosaic cucurbits retained the power of infection for only a short period after their extraction and usually lost their virulence within 24 to 48 hours. Dried material

<sup>4</sup> In this connection Brandes (7, p. 132) credits F. S. Earle with calling attention, in a then unpublished paper, to a method of inoculating with juice expressed under oil to hinder possible oxidation. Bruner (11), however, states it as his understanding that the procedure of extracting the cane juice under mineral oil to avoid oxidation was originally suggested by E. D. Colón and F. A. López Domínguez, of the insular experiment station of Puerto Rico, and that this procedure was employed later by Professor Earle.

<sup>5</sup> This was not intended to imply that the virus would lose its virulence at the end of 24 hours under all conditions, as Doctor Brandes, since his first experiments in 1920, had been storing away samples of extract of sugarcane mosaic for the purpose of testing in the future the effect of age on retention of virulence.

likewise suffered a rapid loss of the infectious power. Preservatives to stop fermentation did not serve to prolong the period of activation in extracted infected juice, and low temperature had only a slight effect in prolonging the power of infection. Henderson and Wingard (18) report that the tobacco ring-spot virus is inactivated in 12 to 24 hours at ordinary room temperature and that they never succeeded in getting infection from dry material, no matter how fresh it happened to be; on the other hand, they found that the virus may retain its virulence for at least 22 months when kept at a temperature of  $-18^{\circ}\text{C}$ .

Allard (1, 2) found that the tobacco-mosaic virus was still infectious after it had been bottled for four months. He later found (2, p. 636) that at the end of 231 days similarly kept extract of tobacco-mosaic virus was still highly infectious.

Earle (16, p. 17) states that—

oxidation might affect the vitality of the mosaic virus, and that a sucking insect flying from a diseased to a healthy plant and again feeding might regurgitate a minute quantity of the diseased juice without having exposed it to the air.

With juice pressed from diseased canes, without oil covering, he inoculated 7 canes by means of a hypodermic needle thrust into the leaf spindle above the terminal bud. After about 6 weeks he observed 2 infected plants, whereas from 10 inoculations made with oil-protected diseased juice he observed 5 infected plants after about 4 weeks. Seven other plants, inoculated in the midribs of young leaves, showed no symptoms of the disease. On the day after the tests with oil-protected juice some of the juice was used in 3 additional cane plants, but no positive infection developed. Because of the imperfectly known technic of inoculation and the utter lack of understanding of host response and reaction, little could be said in favor of one procedure over the other, particularly when there seemed to be a lack of uniformity in the results, even with the method that had been successful in one instance.

At about the same time the writer (30) obtained 2 mosaic infections among 5 cane plants that had been inoculated with air-exposed infected juice. In another similar experiment he obtained 2 infections out of 20 inoculations. In both tests the period between inoculation and the appearance of the first mosaic symptoms was about 14 days. The tests of both Earle and the writer, however, unlike those of Brandes, were performed under conditions that did not preclude the possibility of natural infection by aphids.

Following the studies of Brandes, Earle, and the writer, Bruner (11) attempted to transmit sugarcane mosaic, sometimes through the agency of *Aphis maidis* and sometimes without employing the insect vector; he also considered the possibility that exposure to air might have an inhibiting effect upon the virus in pressed-out sugarcane sap. To reduce the air exposure of the sap intended for inoculation he superimposed a mosaic-infected leaf upon a healthy leaf and, holding the two in close contact by finger pressure, he thrust a fine hypodermic needle rapidly through the infected leaf into the healthy one. In this manner he inoculated a tender leaf, a mature leaf, and an old leaf on each of 100 shoots comprising altogether 23 cane stools of the variety *Cristalina*. The experiment was made November 6, 1920; and in March, 1921, only eight shoots in 3 stools showed infection. Bruner believed that the small percentage of

infection in this experiment might have been due to the fact that his inoculated plants were well advanced in development and the rate of growth was slow. In another experiment (his No. 8) Bruner injected infected juice that had been extracted under a cover of oil into 15 sugarcane plants of the variety Cavengerie, while 15 other plants of the same variety were similarly injected with juice extracted in the air. The operations were performed rapidly and in each series only one minute intervened between extraction and inoculation. In each of the lots 3 plants became infected within about 8 weeks from the time of inoculation. Bruner (11, p. 21) states (translation):

We can conclude that the diseased cane juice extracted without any provision against oxidation will reproduce the mosaic just as the juice extracted under a protective cover of oil if there is no delay in making the inoculation after extracting the juice.

In 1920 Brandes (7) obtained no infection of cane plants of the Lahaina variety when juice from infected leaves, prepared without any oil protection, was rubbed with the fingers into either unbroken or needle-scarified surfaces of young leaves; nor did any infection result when inoculation was accomplished by scarifying the young leaf cells with a sharp needle dipped in the air-exposed mosaic-infected juice. Only 1 infection resulted from 10 inoculations made by numerous needle pricks with the same air-exposed juice. In other tests, where the inoculation methods just mentioned were employed except that the diseased juice was pressed from young stems under mineral oil, there were no infections. When a hypodermic syringe was used, 8 out of 10 inoculations made with oil-protected inoculum were successful, whereas only 2 out of 10 inoculations made with unprotected inoculum were successful. Using absorbent cotton wet with juice that had been pressed (without oil) from leaves and upper joints of diseased Lahaina sugarcane, Kunkel (23) rubbed the inoculum into wounded leaves of healthy Striped-Tip sugarcane plants. He performed 3 experiments, each with 6 plants, but reported no infections except from the third experiment, after a lapse of about 3 months, when 5 of the plants showed mosaic symptoms. From 2 to 3 weeks usually intervenes between the date of inoculation and the first appearance of symptoms of this disease.

McRae and Subramaniam (29), using a needle, pricked juice from freshly crushed mosaic leaves into the leaf sheaths and stems of sugarcane, maize, and sorghum. They reported many successful inoculations, but the proportion of infection to inoculation was highly variable; for instance, out of 16 shoots of the cane variety Coimbatore 213, inoculated with juice from diseased Red Mauritius cane, all showed the disease; but out of 30 shoots of Coimbatore 213 inoculated with juice from Coimbatore 205 only 2 developed the disease. While this variability may perhaps have been due in some degree to differences in host relationships and possible attenuation of the virus in some varieties, it seems probable that the method by which the inoculum was secured and introduced into the healthy tissue was often at fault.

Sein (32), using a fine needle, pricked freshly expressed diseased juice (without any oil covering) into the tender parts of the tightly rolled young leaves of cane shoots and obtained more than 50 successful infections in one experiment with 100 inoculated plants. Subsequently he abandoned the use of extracted sap and adopted the

principles of Bruner's (11) method; a band of expanded mosaic leaf was held tightly wrapped around the stripped cylinder of young, tender leaves of the healthy plant and the pin point was repeatedly thrust through the diseased tissue into the healthy leaves. Out of 100 shoots inoculated in the open field by this method, 94 per cent showed infection. Sein thought that his very fine needle, thrust rapidly through the previously unbroken band of diseased leaf into the still tightly rolled cylinder of healthy young leaves, carried diseased material almost directly into healthy tissue without any considerable exposure of the material to the air. It is apparent, however, that if exposure to air is really detrimental to this virus, the air between the two rather rough leaf surfaces may be sufficient to affect the virulence of the minute quantity of infected juice carried by the needle, no matter how firmly the band of diseased leaf is held by the fingers against the leaf cylinder. The advantage of using a very fine needle, as recommended by Sein, would come from the fact that the very small stab lesion resulting from the puncture would cause very little injury, and that the virus would thus be introduced directly into an environment of living cells.

A study of these recent papers on the artificial transmission of sugarcane mosaic makes it evident that their authors have generally thought that this virus (or at any rate the expressed juice from diseased tissues) becomes greatly weakened or loses its capacity for producing mosaic symptoms if the inoculum is not used very soon after it is prepared, and especially if air has access to it during preparation or in the process of inoculation.

It appears, though it has not been exactly thus stated, that the disease-producing capacity of the virus of sugarcane mosaic depends on its being in intimate and practically uninterrupted association with the living cell contents of its host. No one has as yet recorded the occurrence of infection in healthy sugarcane plants from the application of decayed or dried material from infected sugarcane plants or from air-exposed extract of diseased cane tissue when the extract has been kept a long time at ordinary room temperatures. It is commonly supposed that there are marked differences in susceptibility to visible infection, not only among different varieties of cane at any given stage of growth but also among the several parts or regions of an individual plant. The inoculum from diseased plants has generally been applied to the growing point of the cane plant or the youngest leaves, and it seems to be an accepted conclusion that cane roots are not at all receptive to the introduction of the disease. The capacity of the virus to produce the symptoms of sugarcane mosaic seems to depend on a narrow range of external conditions as well as on the varying internal reactions of the host.<sup>6</sup>

#### EXPERIMENTAL METHODS

The inoculum used in the experiments reported in this paper was obtained from young mosaic-infected suckers which sprang up from time to time from the bases of three mosaic-infected fully grown stools of the sugarcane variety P. O. J.<sup>7</sup> 234. After the mature leaves had been discarded the remaining leafy portions, including the false

<sup>6</sup> The inoculation method employed by McKinney (28) in his work with wheat and rye was tested, but in no instance did the inoculated sugarcane show infection.

<sup>7</sup> Proefstation Oost Java.



stems, of about 10 shoots were cut into  $\frac{1}{2}$ -inch lengths and ground in a meat chopper, slowly, in order to avoid overheating the material. The sharply grooved nut-butter-cutter disk furnished with the chopper was employed. All apparatus used had been sterilized by boiling in hot water. The ground material was received from the chopper in a 20-ml porcelain evaporating dish, most of the liquid being then squeezed out by hand through cheesecloth. The nearly dry pulp was discarded and the green filtrate, which contained much material in suspension, including whole and broken chloroplasts, was stored in vials or flasks, without special precautions of any kind except, in some instances, in regard to temperature.

When a cane shoot was to be inoculated some of this liquid was transferred by means of a pipette to the wedge-shaped opening between the youngest expanded leaf blade and the next younger leaf on the same side, which was still rolled. The blade of the youngest expanded leaf bends sharply away from the vertical axis of the plant, and its base partly clasps the false stem or leaf spindle, thus forming a sort of cup or reservoir that retains the liquid, while a small area of the outer surface of the most recently exposed but still rolled leaf is covered by the liquid. The relations of these two leaves and the expressed juice held between them are shown in Figure 1. A fine needle point (usually No. 216, Special Minuten Nadeln), set into a glass rod which served as a handle, was next passed horizontally, or somewhat obliquely downward, through the liquid and into the submerged area of the still rolled leaf. In addition to the direct downward punctures, several vertical cuts with the needle were made through the leaf tissue in order to allow the contact of the virus with the now severed fine transverse connections of the vascular bundles. This stabbing operation was repeated rapidly about 5 or 6 times, with gentle pressure; each needle thrust formed a new puncture, but all punctures were close together and were covered by the juice in the reservoir. This method of inoculation was found to be suitable for young sorghum and *Digitaria* plants as well as for sugarcane. Inoculations were usually made on a cloudy afternoon, when the light intensity was weak and evaporation was slow.

The inoculated plants stood for many weeks in pots of soil in an ordinary greenhouse, at a temperature varying from about 25° to 30° C., and were frequently inspected. Each inoculated culture had a green label showing how and when inoculation was performed. When mosaic symptoms appeared a red label was attached, bearing the date of the first appearance of the disease. Subsequent notes were added as the malady progressed.

The greenhouse was screened, and no individuals of *Aphis maidis*, the natural transmitter of the disease, were observed in the compartments used or in adjacent ones. As has been said, no special precautions were taken to protect the expressed juice from the air. Nor were any pains taken to use the extract immediately, although many inoculations were made on the same day as that on which the extract was prepared—usually after about 2 to 4 hours. When inoculations were not to be made until the day after the extraction or later, the inoculum was stored in open beakers or vials in an electric refrigerator at a temperature of about 4° C. At this temperature, as well as at higher ones, considerable development of bacteria and other microorganisms was observed after 24 hours, and it was suspected that these

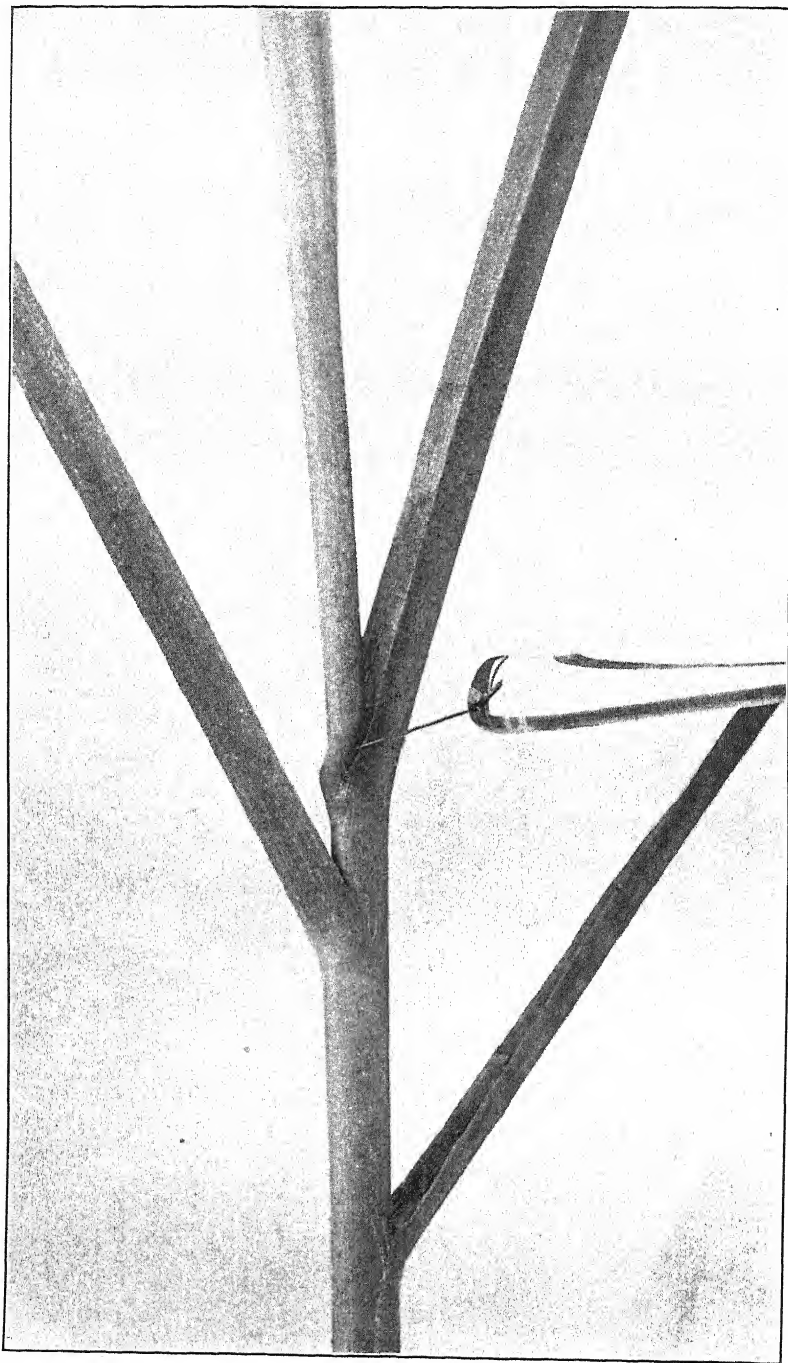


FIGURE 1.—Inoculation of a sugarcane shoot with extracted juice of a mosaic-infected plant. A drop of infected liquid is placed at the wedge-shaped opening between the youngest expanded leaf blade and the next younger, still rolled leaf. The fine needle point is passed through the liquid and into the liquid-covered area of the rolled leaf.

might partly account for some inactivation of the stored extract. Tests in which stored extract was kept at a temperature below the freezing point of water were therefore made. For these tests several somewhat different procedures were tried. (1) Freshly expressed juice from infected cane was kept in open vials in the freezing compartment of an electric refrigerator from within 1 hour after the crushing of the diseased material until the inoculum was used. The temperature of this compartment was  $-6^{\circ}\text{C}.$ , and the liquid became solidly frozen within 24 hours, being thawed at room temperature just before use. (2) Fresh diseased extract in similar open vials was frozen solid within 20 minutes after extraction, by means of blocks of solid carbon dioxide placed alongside in a small, suitably insulated box. The vials of frozen inoculum were then transferred to the refrigerator compartment just mentioned, where the material remained frozen, at about  $-6^{\circ}$  until thawed for use. (3) Fresh juice from mosaic sugarcane was first stored for 6 days at  $4^{\circ}$ ; it was then transferred to the freezing compartment of the refrigerator ( $-6^{\circ}$ ) and stored there until thawed for use.

## EXPERIMENTAL DATA

### INOCULATION TESTS

Data for several hundred inoculation tests made in the spring and summer of 1931 are presented in Table 1. In all these the inoculum employed was juice expressed from mosaic-diseased sugarcane of the variety P. O. J. 234, and the method of inoculation was usually that described in the preceding section. It is, of course, understood that a single inoculation of this kind includes several separate needle punctures, all close together. This type of inoculation is herein designated as the "regular" method. In a few instances other methods of inoculation were used. Tests were made on the Black Amber variety of sorgho and on six mosaic-susceptible varieties of sugarcane, namely: Louisiana Purple, P. O. J. 36-M<sup>8</sup>, D<sup>9</sup>-74, P. O. J. 234, Cristalina, and B. H.<sup>10</sup> 10/12. In each experiment a young, vigorously growing, and apparently healthy plant 1 to 2 feet in height was employed for each inoculation. The results of these tests are shown in Table 1.

Specific records of control plants are omitted, since such records would constitute merely a series of repetitions. In each test, however, control plants as nearly like the inoculated plants as possible were used. The controls received the same treatment as the inoculated plants except that they were not inoculated. The number of controls was usually the same as the number of inoculated plants, but in some instances it was much larger. Several hundred healthy sugarcane plants growing in the greenhouse were continually under observation until March, 1932, and these served as additional, though undesig-nated, controls. None of the uninoculated plants, whether designated as controls or not, showed any mosaic symptom during the period of observation (from April, 1931, to March, 1932). As the records show, however, inoculation did not always produce infection.

<sup>8</sup> Mingka selection of P. O. J. 36.

<sup>9</sup> Demerara.

<sup>10</sup> Barbados hybrid.

TABLE 1.—Results of inoculating Black Amber sorgo and six susceptible varieties of sugarcane with mosaic virus under various conditions in 1932

Variety	Experiment No.	Inoculum		Inoculations			Infections observed		
		Age	Storage temperature	Date	Number	Method	Date	Number	Total
Sorgo (Black Amber)-----	1	Hours 2-4	° C. (a)	Apr. 27	12	(b)	May 11 May 23 May 26 do-----	1 4 3 1	8
	2	24	4	Apr. 28	12	(c)	May 28 May 29 June 4 May 11	2 1 4 1	8
	3	24	4	do-----	12	(d)	June 3 June 3	1 1	2
	4	2-4	(a)	May 27	6	(e)	June 3	0	0
	5	2-4	(a)	do-----	6	(e)	June 12 June 18	1 2	3
Sugarcane:	6	24	4	Apr. 28	6	(c)	May 11 May 15 May 24 May 25 May 28	1 1 2 1 1	5
	7	24	4	May 15	13	(c)	June 1 June 8 June 17 May 28	1 1 4 1	7
	8	24	4	do-----	12	(f)	June 8 June 17 June 17	1 1 1	3
	9	2-4	(a)	May 25	11	(e)	June 7	5	6
	10	2-4	(a)	do-----	10	(e)	June 9	1 0	0
Louisiana Purple-----	11	Days 11	4	do-----	5	(e)	June 8	1	1
	12	10	4	June 23	11	(e)	July 24	3	3
	13	Hours 2-4	(a)	May 27	6	(e)	June 18 June 22 July 17	4 1 1	5
	14	Days 26	4	June 19	6	(e)	July 22 July 23 July 31	1 1 1	3
	15	Hours 2-4	(a)	July 20	30	(e)	Aug. 5 Aug. 6 Aug. 7 Aug. 9 Aug. 11 Aug. 18 Aug. 27 Sept. 22	5 5 1 1 2 2 1 2	20
	16	24	4	July 21	24	(e)	Aug. 5 Aug. 7 Aug. 8 Aug. 19	1 1 1 1	4
	17	Days 14	4	do-----	30	(e)	Aug. 5 Aug. 7 Aug. 11 Oct. 6	2 3 1 3	6
	18	28	a-6	Sept. 17	10	(e)	Oct. 8 Oct. 19 Oct. 1	1 1 1	5
	19	22	b-6	do-----	10	(e)	Oct. 5 Oct. 6 Oct. 7 Oct. 8	2 1 1 1	7
	20	28	c-6	do-----	10	(e)	Oct. 20 Oct. 5	1 1	1

a The inoculum was stored at room temperature.

b The plants were inoculated by rubbing the extract into the young leaves by means of a glass rod.

c The regular method of inoculation was employed (p. 827).

d The plants were inoculated by injecting the extract into a rolled leaf by means of a hypodermic syringe.

e The regular method of inoculation was employed, but the leaf tissue was not punctured.

f The regular method of inoculation was employed except that a coarse needle was used.

g The extract was frozen within 20 minutes after being prepared, previous to storage.

h In storage the extract became frozen within about 24 hours after preparation.

i The extract was stored for 6 days at 4° C., then frozen and stored at -6°.

TABLE 1.—Results of inoculating Black Amber sorgo and six susceptible varieties of sugarcane with mosaic virus under various conditions in 1932—Continued

Variety	Experiment No.	Inoculum		Inoculations			Infections observed			
		Age	Storage temperature	Date	Number	Method	Date	Number	Total	
D-74-----	21	Hours	° C.							
		24	4	June 9	16	(c)	June 22 June 24	1 2	3	
	22	24	4	June 11	8	(c)	June 29 July 3	1 1	2	
	23	Hours	(a)	July 2	6	(c)	Aug. 5	5	5	
	24	Days	2-4							
		28	° -6	Sept. 17	10	(c)	Oct. 5 Oct. 9 Oct. 12 Oct. 20	3 1 1 1	6	
	25	22	h -6	do	10	(c)	Oct. 2 Oct. 7	1 1	2	
	26	28	i -6	do	10	(c)		0	0	
	P. O. J. 234-----	27	28	4	June 22	12	(c)	July 6 July 13 July 15	1 1 1	3
		28	28	4	June 24	11	(c)	July 20	1	1
29		28	° -6	Sept. 17	10	(c)	Oct. 1 Oct. 5	2 1	3	
30		22	h -6	do	10	(c)	Oct. 1 Oct. 5	1 2	3	
Cristalina-----	31	28	i -6	do	10	(c)		0	0	
	32	30	4	June 27	6	(c)	July 11	1	1	
B. H. 10/12-----	33	28	° -6	Sept. 17	10	(c)	Oct. 5 Oct. 6 Oct. 9	2 1 1	4	
							Oct. 1	1		
	34	22	h -6	do	10	(c)	Oct. 5 Oct. 9	2 2	5	
	35	28	i -6	do	10	(c)		0	0	

<sup>a</sup> The inoculum was stored at room temperature.

<sup>c</sup> The regular method of inoculation was employed (p. 827).

<sup>e</sup> The extract was frozen within 20 minutes after being prepared, previous to storage.

<sup>f</sup> In storage the extract became frozen within about 24 hours after preparation.

<sup>h</sup> The extract was stored for 6 days at 4° C., then frozen and stored at -6°.

#### RELATIVE EFFECTIVENESS OF DIFFERENT METHODS OF INOCULATION

Experiment 1, Table 1, shows that sorgo may be successfully inoculated by merely rubbing the extract into the leaves without puncturing the tissue; out of 12 tests, 8 were successful. This rubbing method is not usually so efficient in transmitting the disease, however. Experiments 2 and 3, also with sorgo, show the relatively low degree of efficiency of inoculation by means of a hypodermic syringe as compared with inoculation by means of a drop of inoculum and several fine-needle punctures (regular method). Out of 12 tests, the syringe method gave only 2 infections; the regular method gave 8.

Experiments 4 and 5, also with sorgo, confirm the frequently repeated observation that merely placing inoculum on the surface of the young leaf, without puncturing, is not a successful method of inoculation. In each of these cases there were 6 tests; the regular method gave 3 infections; the same procedure without puncturing gave none. Experiments 9 and 10, with Louisiana Purple sugarcane, show similar results; the regular method gave 6 infections out of 11 inoculations, whereas 10 similar inoculations without puncturing resulted in no infection.

Experiments 7 and 8, with Louisiana Purple sugarcane, indicate that where the regular method of inoculation is employed a very fine needle gives a higher percentage of successful infections than does a coarse dissecting needle. In these two experiments the use of a very fine needle resulted in 7 infections out of a possible 13, whereas when a coarse dissecting needle was employed there were only 3 infections out of a possible 12.

TABLE 2.—*Relative infectiousness of similar mosaic extracts (from infected sugarcane P. O. J. 234) variously treated prior to their use in making inoculations*

Group No.	Treatment of inoculum	Experiment No.	Plant inoculated	Inoculations		
				Num-ber	Num-ber	Per cent
1	Extract stored only about 2 to 4 hours at room temperature.....	5	Sorgo.....	6	3	50
		9	Louisiana Purple cane.....	11	6	55
		13	P. O. J. 36-M cane.....	6	5	83
		15	do.....	30	20	67
		23	D-74 cane.....	6	5	83
	Total.....			59	39	66
2	Extract stored about 1 day at 4° C.....	2	Sorgo.....	12	8	67
		6	Louisiana Purple cane.....	6	5	83
		7	do.....	13	7	54
		16	P. O. J. 36-M cane.....	24	4	17
	Total.....			55	24	44
3	Extract stored 10 to 14 days at 4° C.....	11	Louisiana Purple cane.....	5	1	20
		12	do.....	11	3	27
		17	P. O. J. 36-M cane.....	30	6	20
	Total.....			46	10	22
4	Extract stored 25 to 28 days at 4° C.....	14	P. O. J. 36-M cane.....	5	3	60
		21	D-74 cane.....	16	3	19
		22	do.....	11	2	18
		27	P. O. J. 234 cane.....	12	3	25
		28	do.....	11	1	9
	Total.....	32	Cristalina cane.....	6	1	17
	Total.....			61	13	21
5	Extract frozen within 20 minutes after preparation, then stored about 27 days at -6° C.....	18	P. O. J. 36-M cane.....	10	5	50
		24	D-74 cane.....	10	6	60
		29	P. O. J. 36-M cane.....	10	3	30
		33	B. H. 10/12 cane.....	10	4	40
	Total.....			40	18	45
6	Extract stored about 27 days, at -6° C., becoming frozen within first 24 hours.....	19	P. O. J. 36-M cane.....	10	7	70
		25	D-74 cane.....	10	2	20
		30	P. O. J. 234 cane.....	10	3	30
		34	B. H. 10/12 cane.....	10	5	50
	Total.....			40	17	43
7	Extract first stored 6 days at 4° C., then frozen and stored 20 days at -6°.....	20	P. O. J. 36-M cane.....	10	1	10
		26	D-74 cane.....	10	0	0
		31	P. O. J. 234 cane.....	10	0	0
		35	B. H. 10/12 cane.....	10	0	0
	Total.....			40	1	3

To secure the highest percentage of infection it appears to be desirable to use the extracted juice while fresh. Storage in an ordinary refrigerator, however, may be expected to preserve the infectiousness of this virus for a long time, despite its apparently progressive loss of virulence.

The percentages for groups 5, 6, and 7 indicate that a storage temperature somewhat below the freezing point of water preserved the virulence of the virus much more satisfactorily than did a temperature of about 4° C. As might be expected, for freezing storage it appears to be desirable to bring the extract into the frozen condition soon after its preparation, for a 6-day storage at 4° followed by a 20-day storage at -6° gave very unsatisfactory results (group 7, with mean percentage of only 3). Nevertheless, the percentage value for group 5 (with quick freezing) is only slightly—and probably not significantly—higher than that for group 6 (without quick freezing). It

may therefore be recommended that extract of mosaic-infected sugarcane intended for inoculation tests similar to those of the present study should be prepared at a temperature as low as is feasible (with cold press and vessels) and brought to a freezing storage temperature promptly. It is important to note that extract stored for nearly a month at  $-6^{\circ}$  (groups 5 and 6) gave, on an average, infection percentages (45 and 43), approximately the same as that given by similar extract stored at  $4^{\circ}$  for a single day (group 2, with a mean percentage of 44). Since it appears that the freezing of the extract did not diminish its infectiousness, it might be desirable to employ freezing of the infected tissue to aid in freeing the juice from the cells, as has frequently been done when plant tissues were to be extracted for other purposes. The infected material would thus be first frozen, then thawed, ground, and pressed, and finally stored at a freezing temperature.

Although the rather broad field explored in this preliminary study and the consequent lack of extensive series of strictly comparable data make the application of detailed numerical computations and more precise analytical methods inapplicable, yet it may be interesting to note that the highest infection percentages for individual experiments are shown for experiments 13 and 23 (group 1, with fresh extract) and for experiment 6 (group 2, with extract that had been stored about 1 day at  $4^{\circ}$  C.), each of which gave 5 infections out of 6 inoculations, or 83 per cent of successful transmission of the disease. The next lower individual percentages (67 to 70) were given by experiment 15 (group 1, with fresh extract), experiment 2 (group 2, with extract that had been stored about 1 day at  $4^{\circ}$ ), and experiment 19 (group 6, with extract that had been stored about 27 days at  $-6^{\circ}$ ).

## DISCUSSION

The causative substance or virus of the sugarcane mosaic disease, when separated from the host, seems to be much more sensitive to its surroundings than are some of the other and better known plant viruses, which may be readily and successfully transmitted by the simple operation of rubbing into the bruised tissues of an uninfected plant a minute quantity of material from crushed cells of a diseased plant.

The deterioration of the juice expressed from infected plants is probably the result of a number of factors. When cells are crushed and pressed the physical, chemical, and physiological influences normally prevailing in the living cell contents must undergo great alteration; the characteristic vital coordinations are disturbed and the activities of various catalytic agents, such as the enzymes, become either retarded or accelerated. Furthermore, the relations between the extracted juice and the atmosphere, especially those connected with the interchange of oxygen and carbon dioxide, must be very different from the corresponding relations between living cell contents and the atmosphere. Moreover, bacteria or other microorganisms may become active in the expressed juice.

The many failures that have attended attempts to transmit the sugarcane mosaic by means of expressed sap, even when freshly prepared, indicate that successful transmission may depend on some specific manner of introducing the extracted sap into the healthy plant. The tissue with which the inoculum first comes in contact must serve as a suitable and receptive medium for the continuation



of the life or activity of the infective substance, or it must at least offer a vehicle for the conduction or transfer of that substance to cells that are capable of harboring it and disseminating it through the plant. In his study of the mechanics of inoculation, Brandes (9) pointed out that *Aphis maidis*, the only known natural vector of sugarcane mosaic, apparently causes no perceptible destruction of tissue as it feeds on the leaf of maize, although the fine setae of the insect's proboscis pass through the epidermis of the leaf, and generally past several cell layers until they penetrate the phloem. The slight surface wounding caused by the aphid seems to produce no serious injury, nor do the disease symptoms appear first at the place of puncture. As shown by the writer (30) and others, mere surface contact between healthy and diseased sugarcane plants does not bring about transmission, since leaves of healthy plants in the field in contact with leaves of diseased plants remained healthy indefinitely.

It seems clear that in order to produce infection, virus or infected juice must come into intimate contact with the inner cells. But artificial applications to interior tissues do not always lead to infection. According to Stevenson (37), Earle (16), the writer (30), and Sein (32), the application of diseased tissue or juice to knife-cut surfaces of internal stem tissue of healthy plants or cuttings did not produce the disease. Nevertheless, Bonazzi (5) reported successful inoculations through comparatively large stem wounds made with a borer, and Wilbrink (41) induced infection in healthy seed cuttings by cutting them with a knife that had been used to cut diseased cane. Brandes (9) suggested that in plants inoculated by means of aphids the phloem may be the path of transmission and that in successful artificial inoculations through mature stem tissue the infection may have been transmitted to the new shoots by way of the vascular bundles rather than through the mature and inactive parenchymatous tissue. The success or failure of inoculation experiments with this mosaic may be explained by assuming that infection is most apt to result from artificial introduction of diseased juice if the operation is so performed as to bring the inoculum into immediate contact with some special cells of the vascular bundles without undue injury either to these or to the overlying tissues.

Since this virus is systemic in the infected host plant; since the host with the virus in it continues active existence almost indefinitely, although in many cases small regions of dead or shrunken parenchyma may be observed in the stems of infected plants (30); and since the virus loses its potency in dead tissue, it follows that the virus is not only naturally adapted to the living cells of the host but that its virulence depends upon the conditions of living tissue.

When successful inoculations were made through the rolled leaves of a young cane plant the first visible symptoms of mosaic were generally observed only after two weeks or more, usually as much as 12 inches or more below the point of inoculation and in the second or third leaf, but not in the outermost and oldest leaf of the spindle into which inoculation had been made. At the same time first symptoms were often discernible in inner leaves of the spindle that had not been reached by the infecting instrument at all. The virus must have descended several inches through the first infected older leaf or leaves to their insertions, whence it seems to have passed into the bases of the younger leaves, moving upward with their growth until the symptoms

became visible as the leaves expanded. In this respect sugarcane mosaic is like other mosaic diseases. It may, therefore, be supposed that the virus is not at once active in the production of mosaic symptoms in an inoculated leaf, but that it passes downward in that leaf for a long distance without causing visible injury and produces visible symptoms only after it has moved upward into still younger leaves. This supposition appears to agree with observed facts. There is reason for believing that the path of descent may be in the phloem.

Whatever the method by which diseased sugarcane juice has previously been applied to healthy plants—whether it has been rubbed into wounded leaves, injected with a hypodermic syringe, or pricked in with a needle—no attempt apparently has been made to prevent access of air to the lesions. It is reasonable to suppose that the hydrostatic and gas pressures that prevail in the vessels and in the closed intercellular spaces of the healthy sugarcane leaf are somewhat lower than the gas pressure of the external air and of the substomatal and other gas-filled spaces that are in direct communication with the external air, especially when transpiration is proceeding rapidly. Under such conditions of pressure gradients, minute quantities of air would promptly enter any wound or incision made in the leaf, usually before the entrance of the liquid inoculum, no matter how promptly the latter might occur. Thus it may happen that at least some portions of the internal tissue surfaces suddenly exposed by a mechanical incision become covered with a thin gas layer before they are really reached by the diseased juice, and actual access of the latter to such gas-covered surfaces may be greatly retarded or even prevented. It is believed that these possibilities were largely avoided in the present experiments by first covering the region where inoculation incisions were to be made with a drop of the liquid inoculum, which remained in position as a water seal over the incisions until reduced and removed through evaporation and absorption; suction or diffusion of virus into the wounds continued to occur long after the completion of the stabbing operation. This expedient of making the needle stab through a properly placed drop of diseased juice gave added assurance that the needle point was always thoroughly wetted with the liquid as it entered the foliar tissue.

Because of turgor, the hydrostatic pressure of living cells, including the elements of the phloem, is greater than that of the sap in the vessels; it exceeds also the gas pressure of the atmosphere and of the intercellular spaces. Hence some exudation may occur from living cells that happen to be penetrated by the stabbing needle. When stabbing is performed through a drop of diseased juice the sucked-in inoculum should tend to come into immediate contact with any extruded cell contents, and the complex system of surface-tension gradients thus set up should result in convection currents that would effectively mix the two liquids. This sort of mixing action would be much more rapid than simple diffusion and might continue for a long time after the operation of stabbing had been completed.

#### SUMMARY AND CONCLUSIONS

In this paper the present state of knowledge concerning the mosaic disease of sugarcane is reviewed and new experimental results of inoculation tests with this mosaic are presented. A new method of

inoculation is described. In inoculation tests on healthy plants of the mosaic-susceptible sorgo variety Black Amber and on the mosaic-susceptible sugarcane varieties, the extracted juice of diseased sugarcane was used as inoculum. In making an inoculation a drop of inoculum was placed in the wedge-shaped opening between the base of the youngest expanded leaf blade and the next younger leaf, recently exposed but still tightly rolled, on the same side of the false stem or leaf spindle. Through the liquid held in this wedge-shaped reservoir a very fine needle was repeatedly thrust with gentle pressure, pricking the submerged area of the rolled leaf. The pricking or stabbing was thus done under the liquid, which was allowed to remain in place till it evaporated or was absorbed. Where fresh or properly stored juice was used, high percentages of infection generally resulted within a few weeks, although the juice was never protected from the air. Except in inoculated plants, no infection was observed at any time during the investigation either in plants serving as controls or in other plants in the greenhouse.

The results obtained indicated that expressed juice of mosaic-infected sugarcane tends increasingly to lose its infectiousness if kept in open beakers or vials for a day or more at room temperature or even at a temperature of about  $4^{\circ}$  C. This tendency was largely overcome, however, when the juice was stored in open vessels at a temperature of about  $-6^{\circ}$ , at which the liquid became solidly frozen within 24 hours, and so remained until thawed at room temperature just before it was used in inoculation tests. High percentages of infection were obtained when inoculations were made (by the new or "regular" method) with juice that had been stored in the frozen condition for as long as 27 days. Infectious juice that had been kept for 6 days at a temperature of  $4^{\circ}$  and was then frozen and stored for 20 days at  $-6^{\circ}$  was found to have lost most or all of its infectiousness. In the light of present knowledge it may be recommended that infected juice for inoculation experiments of the sort here reported be frozen as soon as feasible after its preparation and be kept frozen until thawed for use; and also that the grinding and pressing operations by which infected juice is prepared should be performed at a temperature as low as possible.

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# THE EFFECT OF COOKING ON THE VITAMIN A AND C CONTENT OF FRESH AND DRIED APRICOTS<sup>1</sup>

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## INTRODUCTION

The effect of drying on the vitamin content of fruits has been the subject of investigation in the laboratories of household science and fruit products of this station for a number of years. The A and C vitamins have been considered chiefly and the fruits studied were peaches, prunes, and apricots. As previously reported (4, 6)<sup>2</sup> vitamin C is well retained by sulphured, dehydrated, and sun-dried fruit but is largely lost by the unsulphured dried products. In apricots a minimum of 470 parts per million of sulphur dioxide in the dried product was found to be necessary for satisfactory preservation of the anti-scorbutic property. Lye dipping previous to the sulphuring was found to be necessary in the case of prunes in order to produce sufficient penetration of the protective sulphur dioxide to effect the preservation of the vitamin C in the dried product.

Vitamin A was shown (5) to be well retained, 40 to 100 per cent usually, by dried peaches and prunes, both sulphured and unsulphured, and less well preserved in the dried apricots, 16 to 51 per cent. The dried apricots nevertheless were fairly rich in vitamin A because of the unusual endowment of the fresh fruit. Both sulphuring and dehydration were favorable to the retention of this vitamin as well as to that of vitamin C.

The question was raised by Nelson and Jones<sup>3</sup> of the Bureau of Chemistry and Soils, United States Department of Agriculture, as to the applicability of these findings to the processed sulphured dried fruit usually found on the market. They reported that very little vitamin C remained in five samples of processed and resulphured apricots, although the first sulphuring was found to exert a striking preservative effect on this vitamin.

No detail was reported, and in a later summary (3, p. 24) the statement was made that—

sulphured apricots retain their original vitamin C content remarkably well through commercial processing and subsequent storage, whereas dried unsulphured apricots are devoid of this vitamin. However, ordinary methods of cooking destroy the vitamin completely, and if the practice of sulphuring is to be of any practical significance in the preservation of vitamin C it will be necessary to devise other methods of preparing the fruit for the table.

Before the publication of these comments a series of tests had been begun in these laboratories designed to answer both questions raised, namely, (1) the effect of processing and resulphuring and (2) of cooking on the vitamins of sulphured and unsulphured dried apricots.

<sup>1</sup> Received for publication Aug. 8, 1932; issued June, 1933.

<sup>2</sup> Reference is made by number (italic) to *Literature Cited*, p. 849.

<sup>3</sup> NELSON, E. M., and JONES, D. B. THE VITAMIN C CONTENT OF SULPHURED APRICOTS. [Unpublished. Title in abstracts of papers presented at Cincinnati meeting of the Amer. Chem. Soc., 1930.]

## PREPARATION OF THE FRUIT

The fruit was prepared as previously described (6), particular care being taken that the frozen fresh fruit when packed was completely deoxygenated by evacuation and refilling with nitrogen. Royal apricots from the same orchard were used in preparation of samples S1, S2, and S3 (Table 1) and, in preparing S4, fruit from an adjoining orchard was used which had been harvested at the same time. As indicated in Table 1 the last sample was subjected to the usual commercial storage for at least five months and was then processed and resulphured according to current commercial practice.

TABLE 1.—*Preparation and composition of fresh and dried Royal apricots*

Treatment of fruit	Lot No.	Method of preparation	Moisture	Net shrinkage	pH	Sulphur dioxide content
			<i>Per cent</i>	<i>Per cent</i>		<i>Parts per million</i>
Fresh frozen.....	S1	Pitted, ground, evacuated, released with nitrogen twice, and sealed and kept at $-17^{\circ}$ C.	79.4	-----	3.70	-----
Sulphured, sun-dried....	S2	Pitted, sulphured 4 hours, sun-dried 9 days, ground, packed in 8-ounce tin cans, stored at $0^{\circ}$ C.	17.5	4.01	3.40	480
Unsulphured, sun-dried..	S3	Prepared as S2 but without sulphuring.	14.7	4.14	3.75	-----
Processed, sulphured, sun-dried.	S4	Prepared as S2 but held in ordinary storage at room temperature for 5 months, then processed in the usual commercial fashion, ground, packed, and stored as was S2.	24.9	3.65	3.50	1,090

## THE COOKING PROCESS

The cooking of the fresh apricots was carried on in approximately 500-g lots without added water in a covered container over boiling water. The temperature of the fruit was brought to  $85^{\circ}$  C. in 15 minutes and maintained there for 5 minutes, a total cooking time of 20 minutes. The mixture was stirred twice during this interval without removing the cover. The doses of fresh fruit were weighed after cooking. The dried fruit was soaked in a small quantity of warm water for 15 minutes and was then cooked by the same method used for the fresh fruit. All dried-fruit doses were cooked separately in order to eliminate variations in water content. The ground dried apricot cooked in this fashion is quite tender and palatable. This method of brief soaking and rapid cooking of dried fruit has recently been advocated by several workers (10) although somewhat longer periods (15 to 20 minutes total cooking, 3 to 5 of which was at boiling temperature), than those used in this study were found desirable for cooking unbroken apricot halves. Part of the tests were made on dried fruit soaked  $2\frac{1}{2}$  hours in boiling water and then cooked 15 minutes over the open flame. No difference in effect upon vitamin content was noted between this and the other methods used.

## EXPERIMENTAL RESULTS

## THE VITAMIN C TESTS

The methods used in testing for vitamin C were those previously described. The period of feeding was maintained at 60 days, which period this laboratory had found to be as satisfactory for vitamin C assay as 90 days (6).



TABLE 2.—Vitamin C content of raw and cooked fresh apricots

Treatment and crop	Condition	Quantity fed daily	Guinea pigs used	Average weight of animals			Average gain or loss in weight per day	Average length of period	Scurvy score
				Initial	Final	Gain or loss			
Fresh frozen, 1930 crop...	Raw	Grams 10	Number 3	Grams 340	Grams 304	Grams -36	Grams -0.6	Days 56	8
	Cooked	-----	4	343	263	-80	-1.6	49	12
	Raw	15	4	327	508	181	3.2	60	0
	Cooked	-----	7	359	417	58	1.1	51	5
	Raw	20	4	325	488	163	2.8	58	0
	Cooked	-----	5	351	453	102	1.8	56	3
Fresh frozen, 1929 crop...	do.	30	2	352	542	190	3.8	50	0
	Raw	15	3	347	459	112	2.2	51	0
	do.	25	3	324	452	128	2.0	64	0

As shown in Table 2 and Figure 1, 15 g of the fresh raw apricot daily afforded full protection against scurvy as indicated by the usual autopsy findings, but more than 20 g of the cooked fresh fruit was needed for similar protection. There would seem to be a consistent loss of antiscorbutic value in this fruit attributable to the amount of cooking used in these tests. This loss is apparently 30 to 50 per cent of the vitamin C activity of the fresh raw fruit.

Similar cooking of the sulphured dried apricots, S2, brought about less decrease in the vitamin C value. The differences in scurvy scores and in rate of growth, as shown in Table 3 and Figure 2, by the animals fed the same dosages, raw and cooked, are considerably smaller than in the case of the fresh apricots. The sulphur dioxide may exert its protective effect on the vitamin during the cooking as well as during the drying process. Since the raw, unsulphured, dried fruit, as was found in a number of cases pre-

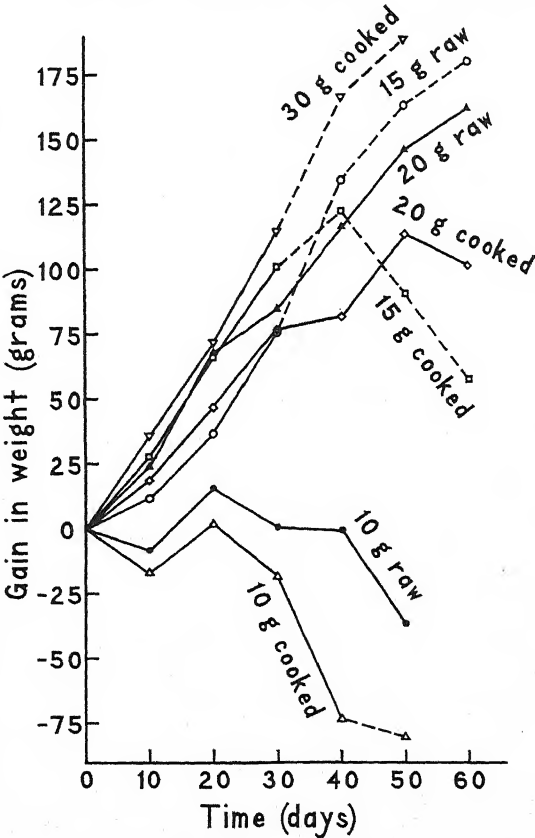


FIGURE 1.—Average growth curves of guinea pigs fed various quantities of raw and cooked fresh apricots, S1. When one or more animals in a group died or was sacrificed the curve is continued as a dotted line

viously, is too poor in the antiscorbutic property to afford any noticeable protection it was not deemed worth while to make the test with cooked unsulphured dried fruit (4).

TABLE 3.—*Vitamin C content of raw and cooked, sulphured and unsulphured, dried apricots*

Treatment and crop	Lot No.	Condition	Quantity fed daily		Guinea pigs used	Average weight of animals			Average gain or loss in weight per day	Average length of period	Scurvy score	Retention of vitamin C of fresh raw fruit (per cent)
			Dried fruit	Equivalent in fresh fruit		Initial	Final	Gain or loss				
			Gms	Gms	Number	Gms	Gms	Gms	Gms	Days		
Sulphured sun-dried, 1930 crop.	S2	Raw	2	8	4	348	305	-43	-0.9	49	8	Raw and cooked, 94-100.
		do.	3	12	5	312	368	56	0.9	60	1	
		Cooked	4	16	3	328	353	25	0.4	56	7	
		Raw	4	16	7	319	428	109	1.8	60	1	
After storage at room temperature for 1 year.	S2	Cooked	5	20	5	329	420	91	1.5	60	1	Raw and cooked, about 40.
		Raw	5	20	4	314	487	173	2.9	60	1	
		Cooked	5	20	3	309	444	135	2.2	60	2	
		Raw	5	20	3	328	253	-75	-1.3	60	8	
Unsulphured sun-dried, 1930 crop.	S3	Cooked	8	32	6	315	257	-58	-1.3	44	9	Raw and cooked, about 40.
Unsulphured dehydrated, 1929 crop.		do.			4	322	339	17	0.3	57	7	
Unsulphured dehydrated, 1928 crop.												
Sulphured sun-dried, processed, 1930 crop.	S4	Raw	10	41	1	310	206	-104	-3.0	34	20	0.
		do.	15	62	3	353	227	-126	-3.8	33	13	0.
		do.	1-10	4.5-45	8	326	230	-96	-3.0	32	20	0.
		do.										
Sulphured sun-dried, processed, 1930 crop.	S4	Cooked	2	7	3	319	217	-102	-3.3	31	9	Raw and cooked, about 50.
		do.			2	342	257	-85	-2.0	43	12	
		Raw	3	11	5	319	235	-84	-2.5	34	16	
		Cooked	4	14	3	353	267	-86	-2.5	35	12	
		Raw	4	14	4	329	263	-66	-2.0	35	12	
		Cooked	4	14	4	328	327	-1	0.0	53	10	
		Raw	5	18	10	329	352	-23	-0.4	57	17	
		Cooked	5	18	2	348	273	-75	-1.4	54	15	
		Raw	8	29	6	338	301	-37	-0.9	60	4	
		Cooked	8	29	4	339	357	18	0.8	60	4	
		Raw	10	36	1	330	437	107	1.8	60	2	

After storage at room temperature for one year the vitamin C content of the sulphured dried fruit, S2, was tested again, both raw and after a longer cooking process. The fruit which had been ground and kept in 8-ounce tin cans, was soaked for 2½ hours in three times its weight of water which was boiling when it was added to the fruit, and was then cooked over the open flame for 15 minutes. The doses could be measured accurately in equivalents of the raw material by comparing the weights of the cooked and raw fruit. As shown in Table 3 a considerable loss of vitamin C occurred during the long storage of this fruit, a loss possibly correlated with the corresponding loss of sulphur dioxide during storage now under investigation in the fruit products laboratory (8).

The same influences which produce oxidation of the sulphur dioxide may operate to oxidize the vitamin. At any rate, after storage at room temperature for one year or more, 5 g of the sulphured, dried apricots either cooked or raw did not protect the animals as nearly completely from scurvy as when the fruit was first tested, but appeared

equivalent antiscorbutically to only 2 g of the original lot. Even 8 g cooked provided only partial protection, about equivalent to that afforded by 3 g of the fruit originally tested. Thus apparently only 40 per cent of the antiscorbutic property was retained after long storage.

The sulphur dioxide content of this stored specimen was tested and found to be only 330 p. p. m. as compared with 480 p. p. m. when the fruit was first prepared. This loss of 31 per cent of the sulphur dioxide may be compared with the loss of 60 per cent of antiscorbutic value. A further test was made of the sulphur dioxide remaining in the stored fruit after the cooking process above described (15 minutes rapid boiling over the open flame). It was found that less than half the quantity present in the raw sample remained, or only 160 p. p. m., a loss of 52 per cent due to cooking. If there is any advantage to be gained by decrease in the sulphur dioxide ingested with dried fruit this decrease during cooking may be looked upon as favorable, particularly since it seems to be accompanied by no concomitant loss of vitamin.

The processed re-sulphured fruit, S4, was found to be less well endowed with vitamin C than the unprocessed specimen S2, although with 8 and 10 g doses both raw and cooked nearly complete protection was afforded. Here again as in S2, shown in Figures 2 and 3 and Table 3, the cooking apparently had little destructive effect upon the vitamin.

Fifteen grams of the raw fresh apricot may be considered the minimum protective dose, and 25 g a reasonable estimate of the minimum dose of the cooked fresh fruit. Since the equivalent of 16 g raw or cooked fed as S2 appears to be the minimum dose of the sulphured dried fruit which furnishes practically complete protection, full retention of the vitamin value of the fresh apricot is shown by both the raw and cooked sulphured dried product.

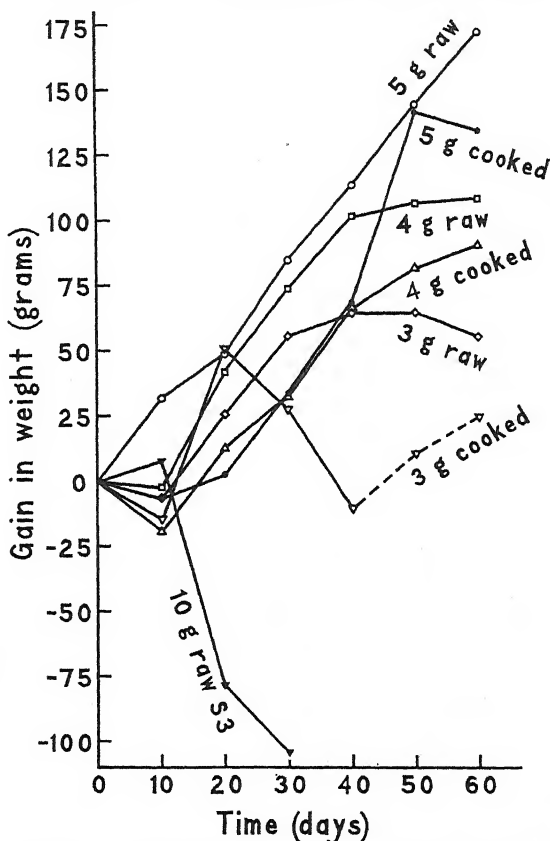


FIGURE 2.—Average growth curves of guinea pigs fed various quantities of raw and cooked sulphured, dried apricots, S2 and raw, unsulphured apricots S3

Since at least 8 to 10 g of the processed fruit, S4, equivalent to 29 to 36 g fresh, must be fed to obtain a fair degree of protection, appar-

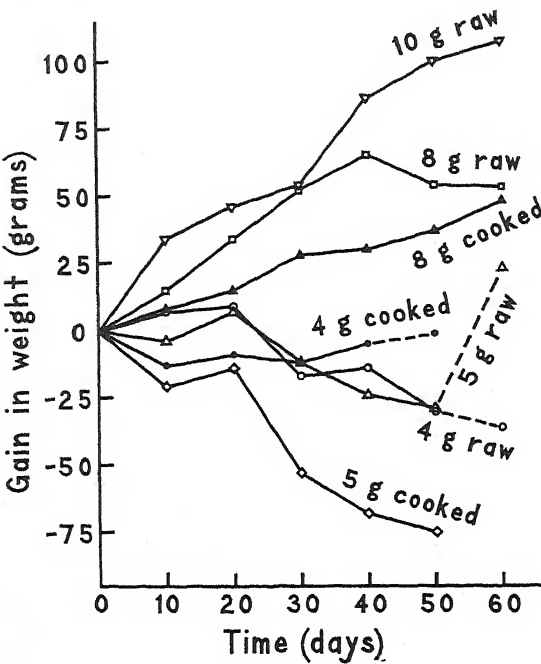


FIGURE 3.—Average growth curves of guinea pigs fed various quantities of raw and cooked processed, sulphured apricots, S4

ently one-half the original antiscorbutic activity of the fresh fruit is lost. The long storage period at room temperature to which this sample had been subjected may be of more importance than the processing in explaining this loss, a supposition borne out by the actual loss in both vitamin C and sulphur dioxide observed in the S2 sample tested after one year in such storage. Storage at 0° C., the ordinary practice for the dried fruit tested in this laboratory, has been found in the case of several kinds of fruit to permit little or no loss of either vitamins C or A.

Freezing storage would therefore appear to be desirable for dried fruits in order to conserve these vitamins.

TABLE 4.—Vitamin A content of raw and cooked fresh apricots

Treatment, crop, and water content	Condition	Quantity fed daily	Rats used	Average weight of animals			Average length of period	Average gain in weight per week
				Initial	Final	Gain		
Fresh frozen, 1930 crop, 79.4 per cent water.	Raw	15	4	73	81	8	24	2.3
	Cooked		6	68	89	21	45	3.2
	Raw	25	4	72	92	20	44	3.2
	Cooked		6	77	113	36	47	5.3
	Raw	30	12	68	105	37	55	4.7
	Cooked		9	54	89	35	45	5.4
	Raw	40-50	9	60	110	50	56	6.3
	Cooked		6	71	133	62	56	7.7
Fresh frozen, 1928 crop, 82.9 per cent water.	Raw	25	3	92	151	59	56	7.3
	do.	50	9	59	131	72	56	9.0
	do.	100	8	71	148	77	56	9.6
	do.	150	3	68	149	81	56	10.2
	do.	200	3	79	181	102	56	12.8

TABLE 5.—Vitamin A content of raw and cooked, sulphured and unsulphured, dried apricots

Treatment and water content	Lot No.	Condition	Quantity fed daily		Rats used	Average weight of animals			Average length of period	Average gain in weight per week	Retention of vitamin A of fresh fruit (per cent)
			Dried fruit	Equivalent in fresh fruit		Initial	Final	Gain			
Sulphured sun-dried, 17.5 per cent water.	S2	Raw	Mg	Mg	Num-ber	Gms	Gms	Gms	Days	Gms	Raw, 26-41. Cooked, 25-33.
		Cooked	22.5	90	4	55	96	41	50	5.7	
		Raw	30.0	120	10	90	141	51	56	6.4	
		Cooked	37.5	150	7	87	145	58	54	7.5	
		Raw	30.0	120	10	117	161	44	53	5.9	
		Cooked	37.5	150	4	75	130	55	56	6.9	
Unsulphured sun-dried, 14.7 per cent water.	S3	Raw	22.5	93	5	71	143	72	56	9.0	Raw, 18-24. Cooked, 9-20.
		Cooked	30.0	124	7	107	134	27	50	3.8	
		Raw	30.0	124	10	111	132	21	48	3.0	
		Cooked	37.5	155	14	102	141	39	55	4.9	
		Raw	30.0	124	10	121	153	32	46	4.9	
		Cooked	37.5	155	3	91	108	17	32	3.8	
Processed sulphured sun-dried, 24.9 per cent water.	S4	Raw	22.5	82	3	109	127	18	53	2.4	Raw, 18-29. Cooked, 18-23.
		Cooked	30.0	109	8	106	122	16	51	2.2	
		Raw	30.0	109	7	110	135	25	56	3.1	
		Cooked	37.5	137	10	99	142	43	55	5.5	
		Raw	30.0	109	8	104	146	42	53	5.6	
		Cooked	37.5	137	9	99	153	54	56	6.8	
Sulphured sun-dried, 1928 crop, 17.5-19 per cent water.	---	Raw	20.0	96	9	105	149	44	56	5.5	Raw, 17-21.
		do	25.0	118	8	69	109	40	56	5.0	
		do	30.0	143	8	87	151	64	56	8.0	
		do	30.0	143	7	69	123	54	56	6.8	

## THE VITAMIN A TEST

The methods used in testing for vitamin A were the same as have been previously used in this laboratory (5). The cooked doses of both fresh and sulphured dried fruit appeared to support somewhat better growth (Tables 4 and 5 and fig. 4) than did the raw. This may be due to better intestinal absorption of the former, possibly through the destruction of the oxidases known to be present in fresh apricots (2) or to some other factor by which better carotene absorption is assured, a process upon which vitamin A utilization appears to depend (1). The vitamin A value of the fresh apricots of the 1930 crop as shown in Table 4 is somewhat lower than that found for the 1928 crop. The sulphured dried fruit, S2, showed values (Table 5) quite similar to those of the corresponding 1928 sample. The processed fruit, S4, appeared to have a slightly decreased vitamin A value and the unsulphured, dried apricot, S3, showed somewhat greater loss when compared with both the fresh and the sulphured dried products. The percentage retention by the dried samples of the vitamin A of the fresh fruit was calculated by comparison of the dose (in terms of fresh-fruit equivalent) required to provide the same amount of growth by the fresh and dried fruit. Thus, since a 90-mg equivalent of S2 raw and 30 mg of fresh raw apricot, S1, allow an average weekly gain of 5.7 and 4.7 g, respectively, S2 may be said to retain about 33 per cent of the vitamin A activity of the fresh apricot. Of course such comparisons can be made only roughly and no claim is put forward for finely drawn distinctions among the percentage retentions given in Table 5. Nevertheless it seems reasonable to assume that 26 to 41 per cent of the vitamin A is preserved in

the raw sulphured sun-dried fruit, S2, and 25 to 33 per cent in the cooked samples of the same lot; 16 to 28 per cent in the raw unsulphured dried sample, S3, and 9 to 20 per cent of the cooked; 18 to 38 per cent in the raw processed sulphured fruit, S4, and 18 to 23 per cent of the cooked. (The cooked fresh fruit is compared with the cooked dried samples and the raw fresh with the raw dried.) The order of decreasing retention value appears to be S2, S4, S3, for both raw and cooked doses as illustrated in Figure 4, and some loss due to cooking appears to occur in the unsulphured lot, S3.

Attention is called to the unusually high vitamin A content of apricots, a characteristic undoubtedly connected with their pigmentation by carotene. A comparison of the carotene and vitamin A values of these apricot samples was made and found to exhibit a close correlation (7). In terms of the Sherman and Munsell (9) units, the raw

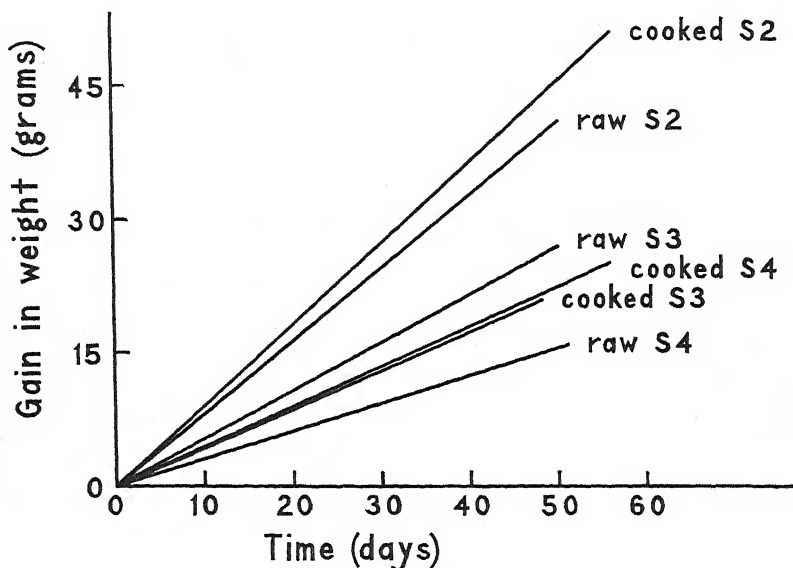


FIGURE 4.—Average weight increases of rats fed 22.5 mg daily of raw and cooked dried apricots: Sulphured sun-dried, S2; unsulphured, sun-dried, S3; processed, sulphured, sun-dried, S4

fresh apricots contain 18,000 units per pound, the cooked fresh apricots, 30,000 units per pound, the sulphured and unsulphured dried fruit about 20,000 units per pound. These values are about the same as those for butter, cheese, cream, and spinach and are exceeded only by liver, cod liver oil, and certain other liver oils.

In terms of the international vitamin A unit recently adopted by the Leagues of Nations Health Organization, 0.001 mg carotene, the fresh apricot, S1, contains 18 to 21, S2 55 and S3, 51 units per gram (7). These calculations are made from the colorimetric determination of carotene in the samples. If the biological test for carotene be used and the growth value of 0.001 mg carotene in vitamin A depleted rats be accepted as 6 to 8 g per week for eight weeks (7) the value for the fresh apricots becomes 22, for the sulphured dried 33, for the unsulphured dried less than 33, and for the processed sulphured dried 27 units per gram. The corresponding figures in units per pound are close to

10,000 for the fresh fruit, 15,000 for S2, something less than 15,000 for S3, and more than 12,000 for S4.

It seems desirable to express the vitamin A value of foods in terms of the international unit but as may be seen in this case it is apparently not yet safe to attempt to use colorimetric instead of biological methods for assay.

#### SUMMARY

The cooking of fresh apricots, preserved by freezing, reduced their vitamin C content approximately 30 to 50 per cent. Similar cooking of sulphured dried apricots of the same origin had less destructive effect upon this vitamin. It is suggested that the sulphur dioxide in the dried fruit may protect the vitamin C during the cooking as well as during the drying process.

The unsulphured dried fruit was again found to be lacking in anti-scorbutic activity, but the dried sulphured product appeared to retain the full vitamin C value of its fresh fruit equivalent.

Commercially stored, processed, and resulphured dried apricots were found to have lost approximately one-half the antiscorbutic property of the fresh fruit but to have retained the remaining vitamin C throughout the cooking process.

Storage of the sulphured fruit at room temperature in closed cans for one year produced a loss of 60 per cent of its vitamin C and 31 per cent of its sulphur dioxide. Soaking this fruit in water for 2½ hours and then boiling it vigorously for 15 minutes made no change in its vitamin C content but occasioned the loss of approximately one-half of its remaining sulphur dioxide.

The vitamin A value of the cooked fresh fruit was found to be somewhat greater than that of the corresponding raw fruit. This is ascribed to better intestinal utilization of the former, possibly because of enzyme destruction or because of better carotene absorption.

Little change in the vitamin A value of the dried fruit samples was brought about by cooking, but the losses caused by the drying process amounted to 59 to 74 per cent in the sulphured, 76 to 82 per cent in the unsulphured, and 71 to 82 per cent in the processed fruit.

Attention is called to the extraordinarily large vitamin A content of both fresh and dried apricots which are equal in this respect to good butter, cheese, cream, and spinach. The number of international units of vitamin A (0.001 mg carotene) per pound is calculated to be about 10,000 for the fresh raw apricots and 12,000 to 15,000 for the dried fruit.

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# INSECTS COLLECTED ON APPLE BLOSSOMS IN WESTERN NEW YORK<sup>1</sup>

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## INTRODUCTION

In a study of insects in relation to the pollination of fruit blossoms in any district, one of the first questions which arises is how many and what varieties of insects are naturally available for this important work. It might be expected that if, in any given area, the number of trees in bloom at any one time is large, the number of individual insects per tree will be smaller than in an area with few trees, unless the district of large orchards happens to be one unusually suitable for the propagation of the species of insects which pollinate the blossoms. It is recognized by horticulturists that a lack of pollinating insects results either in the setting of few fruits or in defective pollination, so that not all five parts of the apple contain seeds, and the fruit is either defective or drops before ripening. It is well known that in many of the commercial fruit areas there is a sparsity of insects. In order to get a more accurate picture of the insect population of one fruit area of New York State, collections were made in the spring of 1931 from apple trees in a section of Monroe County. The results of this work are briefly recorded in this paper.

Monroe County, N. Y., is in about the center of the western New York fruit belt lying along the south shore of Lake Ontario. The part of the county in which collections were made lies in the former glacial-lake bed on flat land, the only variation in relief being that where the southernmost orchards were located the land surface is slightly more rolling. The most dense areas of fruit lie nearest the lake, and within a mile or two of the lake there is a pronounced retardation of the season in spring due to the influence of the large body of water. This is indicated by the considerably later blooming of the trees in orchard A in this work. In the county apple growing predominates, and as a rule the orchards receive excellent care. Because of the high valuation of the land, the amount of waste land is not great, but more waste land is found nearer the southernmost orchards, which are outside the typical fruit belt. Aside from orcharding, mixed farming and dairying are practiced, and near the city of Rochester there is considerable truck farming.

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## METHOD OF PROCEDURE

Collections<sup>3</sup> were made in seven orchards, hereafter referred to as orchards A, B, C, D, E, F, and G. Honeybees were not collected, but all other insect visitors to apple blossoms were taken whenever found, whether or not they may be considered valuable in pollination. Work was begun in each orchard practically as soon as any blossoms were open, and collection each day was begun as soon as insects visited the flowers and ended either with the oncoming of darkness or because of rain or some such meteorological phenomenon which stopped insect flights for the day. No night collections were made, since during the entire period the temperatures at night were too low for insect flight.

Not all the collectors were able to spare time to work during the full period of apple blossoming, and changes were made in the personnel in the several orchards from time to time. Six of the orchards were in bloom at one time, but the one nearest the lake (orchard A) came into bloom just after the trees in all the other orchards had dropped their petals. In orchard A collections were made for two days only, just after the blossoms opened, and to compensate for the shorter time, four collectors worked there the first day and two the second day, until stopped by rain. The number of hours in which collections were made in each orchard was not the same, and the data on the hours of work and the number of collectors are given later in this paper. The total man-hours of collecting for all seven orchards was 459.

Each hour's catch in each orchard was placed in a separate capsule, with a record of the locality and time. In each orchard hourly records were made of temperature, condition of the sky as to cloudiness, approximate wind velocity, and other meteorological phenomena which could be recorded without the use of instruments. It was thus possible in some degree to correlate the prevalence of the different species with conditions existing at the time of the visits to the blossoms. Temperatures were taken in the shade, whereas, of course, the insects were usually flying in the warmth of the sunshine when the sky was clear, but even a record of shade temperature gives some idea of the temperature conditions under which the insects visited blossoms. The estimates of the different collectors regarding light conditions and the degree of cloudiness were too variable to make the data useful in correlations. This is especially to be regretted because of the noticeable effect of sunshine on the flight of insects, especially solitary bees.

After collection was completed the insects were pinned, and with each individual insect was placed a record of the time and place of its capture.

## BRIEF DESCRIPTION OF THE ORCHARDS

The part of orchard A, where the most collecting was done, consists of 7 acres of MacIntosh apple trees. Adjacent on the north are 3 acres of Rome Beauty trees, on the south is a mixed orchard of older trees, and on the west is a larger orchard of older Baldwin trees. The

<sup>3</sup> The collections were made by W. E. Blauvelt, F. G. Butcher, Chit-tung Chen, P. W. Claassen, C. R. Crosby, G. F. MacLeod, W. D. Mills, W. A. Rawlins, G. H. Rea, G. W. Simpson, D. O. Wolfenbarger, and the author.

trees are in excellent vigor. The set of fruit in 1931 was rather light, except on trees adjacent to the Rome Beauty trees, which came into bloom before the petals had fallen from the MacIntosh trees. The south side of the MacIntosh block bore a heavy crop in 1930, which may have reduced the crop of 1931. In the MacIntosh block insects (including honeybees) were rather scarce during the first part of the blooming period, although nectar was observed in the blossoms. Later on the honeybees were observed to be working well. Large branches from blossoming Oldenburg trees were placed in this orchard to provide pollen for the cross-pollination of the MacIntosh blossoms, and during the two days of collection in this orchard insects were more abundant on these branches than on the trees. During the first day a heavy catch of *Halictus provancheri* was made from one side of an Oldenburg tree in the old orchard to the south. There is little waste land near this orchard. This orchard is in the northwest corner of the county within sight of the lake.

Orchard B consists of 10 acres of mixed varieties (19 in all), not in the best of vigor. The trees are about 20 years old. About one fourth of the trees did not bloom in 1931, and about half bore practically no fruit. The set of fruit on some trees was good. Part of the orchard appears to be poorly drained, and there was small terminal growth. Control of injurious insects is not especially good. Directly across the road from this orchard is a neglected pear orchard in old sod, only a few trees of which bore fruit in 1931. This block of pear trees was visited abundantly by *Eristalis arbustorum*, and the pear blossoms appeared to attract these insects more than did the apple blossoms across the road which were in bloom at the same time. The insects had been breeding in a polluted ditch along the roadside between the two orchards. There is some waste land and meadow land near this orchard, which is in the northwest corner of the county about 2 miles from the lake.

Most of the collecting in orchard C was done in a block of about 15 acres of old trees, the varieties being Baldwin, Rhode Island Greening, Twenty Ounce, and Oldenburg. Adjacent to the block of old trees is a block of about 25 acres of younger trees in which some collections were made. Drainage is poor, and insect and disease control is not satisfactory, being attempted solely by the applications of dusts. The set of fruit in the old orchard was good and in the young orchard only fair. A few bouquets of blossoms of other varieties were used in this orchard but showed no local effect on the set of fruit. Surrounding these orchards are many other blocks of apple trees, the orchards being located in the midst of abundant plantings. There is little waste land, but there is a wood lot to the north of the young orchard. This orchard lies east of orchard B and is about  $1\frac{1}{2}$  miles from the lake.

Orchard D consists of two blocks on opposite sides of the road, separated by cultivated fields. In one block is a solid planting of Northern Spy about 20 years old, in good vigor and well sprayed. In this block the blossoms were pollinated by hand and the set of fruit in 1931 was good, but this can not properly be attributed to insect visitors. Adjacent on the east is a block of woodland, and on the south are open fields, partly under cultivation. The other block consists of Baldwin trees about 50 years old, the block being somewhat

thinned by loss of trees. There is an adjoining wood lot on the west and some open fields near by. More collections were made in the Baldwin block than from the Northern Spy. The set of fruit on the Baldwin was good in 1931. This orchard lies south of orchard C and is about  $2\frac{1}{2}$  miles from the lake.

Orchard E was merely a row of eight apple trees along a roadside, south of the densely planted fruit area. The trees, which are in a totally neglected condition and in poor vigor, are surrounded by meadows and open fields with wide fence rows. Within a mile in each direction there were not to exceed 25 apple trees and not much other attraction for the insects that habitually visit blossoms. This group of trees was selected for the purpose of comparing their condition with that of trees in the typical fruit area. These trees probably did not produce a perfect apple in 1931. This group of trees is about 15 miles directly south of orchard D.

Orchard F consists of about 5 acres of mixed varieties, interplanted. The trees are in good vigor and color and are making good growth. The Northern Spy and Baldwin trees in this orchard did not bloom, but other varieties bloomed abundantly. The orchard is surrounded by woods, waste land, open fields, and other fields under cultivation. There is an old orchard of a few trees adjacent, from which collections were not made. Orchard F lies outside the fruit area and was selected for this reason. The set of fruit in 1931 was good for the varieties which bloomed. This orchard is 2 miles south of orchard E.

Orchard G consists of about 50 acres, about 20 acres of which are Rhode Island Greening, with only occasional other varieties interspersed. The remainder consists of interplanted Baldwin and MacIntosh trees. The trees were formerly neglected and were in poor vigor but have recently been brought to much better condition. This commercial orchard was selected because there are no other orchards near it, and there is considerable waste land, swamp land, and woodland near by. These trees bore heavily in 1930, which may have had some effect on the 1931 crop. This orchard was remarkable for the sparsity of all insects in the spring of 1931, only 32 insects being taken in 53 hours of collection, 3 of which are valueless for pollination. Honeybees were not abundant but doubtless outnumbered all other insects 100 to 1. The Baldwin trees bore a fair set of fruit on those trees that were in good vigor. The set on MacIntosh and Rhode Island Greening trees was practically nothing, except for a few apples on a small number of Rhode Island Greening trees which immediately adjoined some Rome Beauty trees that bloomed at the same time. This orchard is in the southeastern part of the county and outside the fruit belt.

#### INSECTS COLLECTED

While the weather during the apple-blossoming period was cold at night and rain interfered with insect flight on some days, there were also some days and parts of days which were excellent for insect flight. In general there were no unusual weather conditions during this season, so that the small total number of insects captured can not be attributed to unfavorable weather.

The outstanding result of this work was the small number of individual insects captured. While the several orchards differed in the number of insects and in the relative abundance of different species,

in none of them was the insect population abundant. This applied to the orchards in the typical fruit area to a more marked degree than to those south of the fruit area, but orchard G, also south of the fruit belt, was the least well provided with wild insects.

In the 459 man-hours of collection only 3,555 individuals were taken, an average of only 7.75 insects per man per hour. The number of identified species of insects is 145, but a number of specimens were taken for which specific determinations are not now available. This may be considered as a fair representation of the number of species but not of the number of individual insects. During many hours of the collecting period it was impossible to find a single insect on the apple blossoms, their absence being due either to cool weather, lack of sunshine, or to the scarcity of the insects. Although this was the first year in which systematic collections were undertaken in Monroe County, yet from the casual observations of earlier years and from the general experience of those who have visited these orchards, the small number of insects collected is not surprising.

The most abundant wild insects visiting apple blossoms were flies (Diptera) and solitary bees (Hymenoptera). In the collections of 1931 the Diptera somewhat outnumbered the Hymenoptera, as will be seen from the tables which follow. In addition, some Lepidoptera were taken, chiefly of one species, and there were also some Coleoptera and other insects which play little, if any, part in pollination. The Lepidoptera, Coleoptera, Hemiptera, and Neuroptera are not here recorded. While probably all the Hymenoptera, with the exception of the wasps, are helpful in pollination, this is not true of all the Diptera. Some of the smaller flies can not be considered as valuable for pollination. Of the 2,056 Diptera captured, probably at least 200 individuals are too small to be of much value to the fruit grower.

#### DIPTERA

In Table 1 are listed all Diptera captured, and for each species is given the number of individuals taken in each orchard. The prevalence of *Eristalis arbustorum* has already been mentioned in the description of orchard B. A considerable number of individuals of this species are listed in the table under "Miscellaneous," these being the flies taken in the pear orchard across the road from orchard B. If individuals of *Eristalis* had been taken as often as possible, the number for orchard B would have been many times as great, but for most of the collecting period an effort was made to find other species.

TABLE 1.—*Diptera collected and frequency of each species, by orchards*

Species and sex	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
<i>Aedes</i> sp.	1			3		5			9
<i>Campitocladius byssinus</i>			1		1	7			9
<i>Psectrocladius</i> sp., females			2	1					3
<i>Cricotopus bicornatus</i>					1				1
<i>Cricotopus trifasciatus</i>					2	1			4
<i>Tanytarsus</i> sp.			1						1
<i>Chironomus aberrans</i> , females					2	5			7
<i>Chironomus</i> sp., females	4		8	1		33			46
<i>Chironomus</i> sp., males			2			18			20
<i>Chironomus</i> spp.			5	4	4	3			16
<i>Sciara</i> sp., females			6		1	15			22
<i>Sciara</i> sp., males			1			1			2

TABLE 1.—*Diptera collected and frequency of each species, by orchards—Continued*

Species and sex	Frequency of each species by orchards indicated							Mis- cella- neous	Total
	A	B	C	D	E	F	G		
<i>Bibio ranthopus</i> , females	4	1	1		1				7
<i>Scatopsa notata</i>		1							1
<i>Simulium venustum</i>			2	2	1	2			7
<i>Stratiomyia</i> sp.						1			1
<i>Tabanus lasiophthalmus</i>						3			3
<i>Rhamphomyia</i> spp.				1	6				7
<i>Toxomerus marginatus</i> , females	2	2	1						5
<i>Toxomerus marginatus</i> , males			1						1
<i>Pipiza pisticoidea</i>			1			1	1		3
<i>Platychirus hyperboreus</i> , females					1				1
<i>Platychirus hyperboreus</i> , males				1					1
<i>Platychirus peltatus</i> , males							1		1
<i>Melanostoma mellinum</i> , females					1				1
<i>Melanostoma obscurum</i> , females	2		3	18		4			27
<i>Melanostoma obscurum</i> , males				2					2
<i>Syrphus americanus</i> , females		2	10	2	5	1			20
<i>Syrphus americanus</i> , males	1	1							2
<i>Syrphus arcuatus</i> , females			1	1		1			3
<i>Syrphus arcuatus</i> , males			1						2
<i>Syrphus ribesii</i> females	2		9	3	3	5	1	1	24
<i>Syrphus ribesii</i> , males				2					2
<i>Syrphus torvus</i> , females	1	4	16	7	4	6	3	1	42
<i>Syrphus torvus</i> , males			12	15	3	7	1		39
<i>Allograpta obliqua</i> , females	2	1	1	2	1				7
<i>Sphaerophoria cylindrica</i> , females	4	7	12	6	2	19	2	1	53
<i>Sphaerophoria cylindrica</i> , males	10	6	15	6		21		4	62
<i>Eristalis arbustorum</i> , females	9	172	80	6	2	10	1	151	431
<i>Eristalis arbustorum</i> , males	15	111	65	15		5		102	313
<i>Eristalis bastardi</i>	2		5	1					8
<i>Eristalis dimidiatus</i>		1	2	2		3	1		9
<i>Eristalis flavipes</i>	3			2					5
<i>Eristalis tenax</i>	5	2	3		1	2		1	14
<i>Helophilus hamatus</i> , females	1					1			2
<i>Helophilus similis</i> , females		2	1	2				2	7
<i>Syrirta pipiens</i> , females			1			1			2
<i>Syrirta pipiens</i> , males			2			1			3
<i>Criorhina verbosa</i> , females						1			1
<i>Myoga vesiculosa</i>					2	1			3
<i>Tachinidae</i> spp.	1	1	10	2	17	9	1		41
<i>Sarcophaga</i> sp.	1	3	10	2	10	11		1	38
<i>Phormia regina</i>					4	15			19
<i>Protophormia terrae-novae</i>					1				1
<i>Cyanomyia cadaverina</i>	2		1		1	1			5
<i>Calliphora vomitoria</i>			1	1	1	3			6
<i>Lucilia caesar</i>	3	1	1	1	1				7
<i>Lucilia sericata</i>				2	1	2			5
<i>Pollenia rudis</i>	1	1	44	6	13	34	1	2	102
<i>Cryptolucilia caesarion</i>			1		1				3
<i>Pyrellia cyanicolor</i>				3	1	17			21
<i>Morellia micans</i>			1		5	6			12
<i>Muscina assimilis</i>							1		2
<i>Muscina stabulans</i>			2	1	9	3			15
<i>Myospila medilabunda</i>		1	2		10	4			17
<i>Fannia canicularis</i> , females			7		2	9			18
<i>Fannia canicularis</i> , males		3	14		1	10	1	1	30
<i>Hydomyia brassicae</i> , males	1	5	47	6	5	7		1	72
<i>Hydomyia calicrura</i> , males	5	5	17	9	9	32	2	2	81
<i>Hydomyia</i> sp., females	5	3	81	22	15	35	4	9	174
<i>Hydomyia</i> sp., males	3		21	3	3	11		4	45
<i>Pegomyia affinis</i> , males						1			1
<i>Scatophaga furcata</i> , females			2			1	1	1	5
<i>Scatophaga furcata</i> , males				3					3
<i>Scatophaga stercoraria</i> , females			4			1		1	6
<i>Scatophaga stercoraria</i> , males			4	2	2	1		1	10
<i>Sphaerocera pusilla</i>					1				1
<i>Sphaerocera subsullans</i>						7			7
<i>Leptocera</i> sp.						2			2
<i>Lonchaea polita</i>	1		3			1		1	6
<i>Sepsis pectoralis</i>			2			1			3
<i>Sepsis</i> sp., females			3			1			4
<i>Nemopoda cylindrica</i>						3			3
<i>Piophilus</i> sp.			1		3			2	6
<i>Chloropisca glabra</i>	2								2
<i>Crassiseita costata</i>						5			5
<i>Botanobia nitidissima</i>						2			2
<i>Mallochiella glabra</i>			3		1	6			10
Total	95	337	553	169	161	430	22	289	2,056

Of the Diptera found on apple blossoms the syrphid flies (Syrphidae) are most valuable, and 1,095 individuals of this family are included in the collection. These flies are fairly well distributed in the different orchards, except in orchard E and in orchard G in which, as already mentioned, there was a general lack of all insects.

No effort has been made to list the Diptera according to the time of day and temperature conditions at the time of capture, as is done later for the solitary bees. Flies may be taken for many more hours of the day than solitary bees, because the flies do not return to nests at night or during inclement weather. At cool or cold temperatures they are harder to find and are inactive; so that while they may often be taken at lower temperatures, this does not imply effective work in pollination except at temperatures suitable for flight. It was noted in orchard B that *Eristalis* abandoned the blossoms when the wind was rather strong and was not often seen at the lower temperatures which occurred during the blooming period.

## HYMENOPTERA

For the solitary bees, which are often so important in the pollination of fruit blossoms, certain additional information is of interest. During the progress of collecting, it was noticed that the solitary bees ceased their visits to the blossoms rather early in the day, not being taken abundantly after about 3 p. m. Data for each species of the two more important genera, *Halictus* and *Andrena*, showing the distribution by orchards (as for the Diptera), the lowest temperature at which captures were made, and the latest hour of the day when they were caught the average temperature, and time are given in Tables 2 to 5. The tables show that *Halictus* stops work earlier than does *Andrena*, perhaps because of the smaller body size of the individual bees of the genus *Halictus*. It was found as the work proceeded that in late afternoon only the larger bees were to be seen.

TABLE 2.—Insects of the genus *Halictus* collected and frequency of each species by orchards

Species of <i>Halictus</i>	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
<i>arcuatus</i> .....			1		1	13			15
<i>brycincti</i> .....	3	5	3	1	10	3		2	27
<i>caeruleus</i> .....						1			1
<i>coriaceus</i> .....		3	3	2	5	6		1	20
<i>divergens</i> .....				1	8				1
<i>lerouirii</i> .....	6	9	4	3	8	25		2	62
<i>lineatulus</i> .....	3		2	15	31	11	1		63
<i>nubilis</i> .....				2					2
<i>nymphacarum</i> .....	1		1		1	2			5
<i>obscurus</i> .....						15			15
<i>pilosus</i> .....		1					2		3
<i>proancheri</i> .....	88	14	20	10	99	34		3	268
<i>quebecensis</i> .....			2	5		1			1
<i>sparsus</i> .....			1	4	12	6			13
<i>versatus</i> .....	1		1	4	8	7			24
( <i>Chloralictus</i> ) sp. ....	2	1	2					1	29
Total.....	104	33	39	56	175	130	3	9	549

TABLE 3.—Temperature and time data in regard to the collecting of the genus *Halictus*

Species of <i>Halictus</i>	Temperature		Last hour collected <sup>a</sup>	Hour when most abundant	Average time collected
	Lowest	Average			
	° F.	° F.			
<i>arcuatus</i> .....	59	70	1.30 p. m.	1.30 p. m.	12.00 m.
<i>brycincl</i> .....	52	66	1.00 p. m.	12.00 m.	10.30 a. m.
<i>caerulus</i> .....	71	.....	12.30 p. m.	.....	.....
<i>coriaceus</i> .....	55	65	3.30 p. m.	10.00 a. m.	11.30 a. m.
<i>divergens</i> .....	66	.....	12.00 m.	.....	.....
<i>lerouzi</i> .....	55	69	3.30 p. m.	1.00 p. m.	11.30 a. m.
<i>lineatulus</i> .....	60	67	2.00 p. m.	1.00 p. m.	12.00 m.
<i>nubilus</i> .....	68	.....	2.00 p. m.	.....	.....
<i>nymphaearum</i> .....	59	.....	2.00 p. m.	.....	.....
<i>obscurus</i> .....	63	69	10.30 a. m.	8.30 a. m.	9.15 a. m.
<i>pilosus</i> .....	57	.....	2.00 p. m.	.....	.....
<i>proancheri</i> .....	48	73	<sup>b</sup> 4.30 p. m.	10.00 a. m.	11.00 a. m.
<i>quebecensis</i> .....	83	.....	2.30 p. m.	.....	.....
<i>sparvus</i> .....	65	70	2.30 p. m.	2.00 p. m.	12.30 p. m.
<i>versatus</i> .....	60	66	2.00 p. m.	10.00 a. m.	11.15 a. m.
( <i>Chloralictus</i> ) sp.....	52	.....	3.00 p. m.	.....	.....

<sup>a</sup> Collections were kept separate hour by hour. The time indicated is the beginning of the hour. The hours of collecting began either on the even hour or the half hour.

<sup>b</sup> This bee was collected at an air temperature of 83° F., with bright sunshine. Omitting this individual, the last hour of collection for this species was 2.30 p. m.

TABLE 4.—Insects of the genus *Andrena* collected and frequency of each species by orchards

Species of <i>Andrena</i> and sex	Frequency of each species by orchards indicated							Mis-cellaneous	Total
	A	B	C	D	E	F	G		
<i>carlini</i> , females.....	.....	1	1	11	23	38	.....	.....	74
<i>carlini</i> , males.....	.....	1	7	3	1	3	.....	.....	15
<i>claytoniae</i> , females.....	.....	.....	.....	.....	1	4	.....	.....	5
<i>claytoniae</i> , males.....	.....	.....	1	1	.....	.....	.....	.....	2
<i>crataegi</i> , females.....	.....	.....	.....	.....	.....	2	.....	.....	2
<i>crataegi</i> , males.....	.....	.....	.....	.....	.....	15	.....	.....	15
<i>comoda</i> .....	.....	.....	.....	.....	1	2	.....	.....	3
<i>flavoclypeata</i> , females.....	.....	1	.....	18	9	32	.....	.....	60
<i>flavoclypeata</i> , males.....	.....	.....	.....	.....	.....	2	.....	1	3
<i>forbesi</i> , females.....	.....	1	2	15	28	18	.....	.....	64
<i>forbesi</i> , males.....	1	.....	6	12	2	2	.....	.....	23
<i>hippotes</i> , females.....	2	17	9	10	16	18	.....	.....	72
<i>hippotes</i> , males.....	1	2	2	14	2	4	.....	1	26
<i>mandibularis</i> , females.....	.....	1	.....	9	4	17	.....	.....	31
<i>mandibularis</i> , male.....	.....	.....	.....	.....	.....	1	.....	.....	1
<i>milwaukeeensis</i> .....	.....	.....	.....	31	.....	.....	.....	.....	31
<i>multiplicata</i> .....	.....	1	.....	3	.....	.....	.....	1	5
<i>nivalis</i> , females.....	.....	.....	.....	.....	.....	5	.....	.....	5
<i>nivalis</i> , males.....	1	1	3	14	.....	3	.....	.....	22
<i>personata</i> , female.....	.....	.....	.....	1	.....	.....	.....	.....	1
<i>placida</i> , females.....	1	.....	.....	3	1	4	2	.....	11
<i>rugosa</i> , females.....	.....	.....	.....	4	1	1	.....	.....	6
<i>rugosa</i> , male.....	.....	.....	.....	1	.....	.....	.....	.....	1
<i>vestita</i> , females.....	.....	.....	.....	1	10	22	.....	.....	33
<i>vestita</i> , males.....	3	.....	1	3	5	20	.....	.....	32
<i>vicina</i> , females.....	.....	1	.....	8	18	12	1	.....	40
<i>vicina</i> , males.....	.....	.....	3	2	.....	2	.....	.....	7
<i>wilkella</i> .....	6	5	3	1	1	4	.....	.....	20
<i>Andrena</i> spp.....	.....	.....	2	3	1	4	.....	.....	10
Total.....	15	32	40	168	124	235	3	3	620



TABLE 5.—*Temperature and time data in regard to the collecting of the genus Andrena*

Species of <i>Andrena</i> and sex	Temperature		Last hour collected <sup>a</sup>	Hour when most abundant	Average time collected
	Lowest	Average			
	° F.	° F.			
<i>carlini</i> , females.....	48	64	4.30 p.m.	1.00 p.m.	12.30 p.m.
<i>carlini</i> , males.....	53	61	2.00 p.m.	10.00 a.m.	11.00 a.m.
<i>claytoniae</i> , females.....	60	-----	1.30 p.m.	-----	-----
<i>claytoniae</i> , males.....	60	-----	12.00 p.m.	-----	-----
<i>crataegi</i> , females.....	66	-----	12.30 p.m.	-----	-----
<i>crataegi</i> , males.....	59	67	2.00 p.m.	10.00 a.m.	11.00 a.m.
<i>comoda</i> .....	59	-----	3.00 p.m.	-----	-----
<i>flavoclypeata</i> , females.....	59	68	3.00 p.m.	1.00 p.m.	11.45 a.m.
<i>flavoclypeata</i> , males.....	66	-----	10.30 a.m.	-----	-----
<i>forbesi</i> , females.....	49	65	3.00 p.m.	12.00 m.	12.00 m.
<i>forbesi</i> , males.....	56	64	2.00 p.m.	2.00 p.m.	12.00 m.
<i>hippotes</i> , females.....	46	66	3.00 p.m.	1.00 p.m.	11.30 a.m.
<i>hippotes</i> , males.....	54	63	2.00 p.m.	2.00 p.m.	11.20 a.m.
<i>mandibularis</i> , females.....	57	67	3.00 p.m.	10.00 a.m.	11.45 a.m.
<i>mandibularis</i> , male.....	67	-----	8.30 p.m.	-----	-----
<i>milwaukeeensis</i> .....	61	66	4.00 p.m.	2.00 p.m.	1.25 p.m.
<i>multiplicata</i> .....	62	-----	10.00 a.m.	-----	-----
<i>nivalis</i> , females.....	58	-----	4.30 p.m.	-----	-----
<i>nivalis</i> , males.....	54	62	2.00 p.m.	9.00 a.m.	11.15 a.m.
<i>personata</i> , female.....	59	-----	1.00 p.m.	-----	-----
<i>placida</i> , females.....	63	-----	2.00 p.m.	-----	-----
<i>rugosa</i> , females.....	60	-----	4.30 p.m.	-----	-----
<i>rugosa</i> , male.....	59	-----	1.00 p.m.	-----	-----
<i>vestita</i> , females.....	48	67	2.30 p.m.	10.30 a.m.	11.00 a.m.
<i>vestita</i> , males.....	60	69	2.00 p.m.	10.00 a.m.	10.30 a.m.
<i>vicina</i> , females.....	48	63	2.30 p.m.	1.00 p.m.	12.00 noon.
<i>vicina</i> , males.....	54	-----	4.30 p.m.	-----	-----
<i>wilkella</i> .....	54	75	3.30 p.m.	10.00 a.m.	11.00 a.m.
<i>Andrena</i> spp.....	55	-----	2.00 p.m.	-----	-----

<sup>a</sup> Collections were kept separate hour by hour. The time indicated is the beginning of the hour. The hours of collecting began either on the even hour or the half hour.

An outstanding difference between species of *Halictus* and *Andrena*, in comparison with the *Diptera*, is the fact that the solitary bees build nests and return to them not only to supply the nests with food but also for shelter at night and in bad weather. This doubtless accounts for their absence from the orchards in cold weather and in late afternoon.

A separate tabulation is made for the eight species of bumblebees (119 individuals) taken. (Table 6.) The fact that eight species were taken indicates that the catch was fairly representative. At the time of apple blossoming, only queen bumblebees are to be found, for it is at just this time of year that the queens are seeking locations for their nests or are constructing the very beginnings of the nests. Several queen bumblebees were seen searching for nesting places while this work was under way. Because of the noise which they make when they fly, bumblebees usually receive credit for more effective work in orchards than seems to be due them. Not only are they not abundant at this season, but they often come darting into an orchard, visit two or three flowers, and then depart. Because of their methods of work, they are unusually effective in pollinating those blossoms that they visit. While bumblebees are credited with a fair degree of constancy in their visits to a single species on one trip, their wide range of flight would seem to reduce their value to the fruit grower. The noise which bumblebee queens made as they entered the orchards where collections were being made may have attracted the attention of the collectors and caused a larger number to be taken.

There was in fact some rivalry among the collectors as to which could capture the most bumblebee queens, so that the number is doubtless a little large for these species.

TABLE 6.—*Solitary bees in genera other than Halictus and Andrena, bumblebees, and Hymenoptera other than solitary bees and bumblebees collected and frequency of each species by orchards*

#### OTHER SOLITARY BEES

Species	Frequency of each species by orchards indicated							Miscellaneous	Total
	A	B	C	D	E	F	G		
<i>Agapostemon (Halictus) radiatus</i> .....	1	1							2
<i>Agapostemon taranus</i> .....	1				5	7			13
<i>Augochlora confusa</i> .....			1	1		3			21
<i>Ceratina calcarata</i> .....		1							1
<i>Ceratina dupla</i> .....		1							1
<i>Colletes inaequalis</i> .....						1			1
<i>Nomada obliterata</i> .....	1								1
<i>Osmia atriventris</i> .....			2			2			4
<i>Osmia lignaria</i> .....	1			1	1		1		5
<i>Osmia pumila</i> .....			3					1	5
<i>Sphecodes ranunculi</i> .....	2	2	1	5		1			11
Total.....	6	5	8	7	22	15	1	1	65
Total for all solitary bees.....	125	70	87	231	321	379	7	13	1,233

#### BUMBLEBEES

<i>Bombus affinis</i> .....							1		1
<i>Bombus bimaculatus</i> .....		1		2	4				7
<i>Bombus ferridus</i> .....	2	4	2	3	5	2			18
<i>Bombus impatiens</i> .....	1	5	5	13	2	14		1	41
<i>Bombus perplexus</i> .....		2	1	5		12			20
<i>Bombus ternarius</i> .....						1			1
<i>Bombus terricola</i> .....		4	3	4	2	5	1		19
<i>Bombus vagans</i> .....	2		2	4	1	2		1	12
Total.....	5	16	13	31	14	36	2	2	119

#### MISCELLANEOUS HYMENOPTERA

<i>Ophion bilineatum</i> .....								2	2
<i>Ephialtes</i> sp.....		1	3						4
<i>Amblyteles atrox</i> (?).....			1						1
<i>Amblyteles</i> sp.....			1			1			2
<i>Chrysis coerulans</i> (?).....				1					1
<i>Polistes pallipes</i> .....		1							1
<i>Vespa (Dolichovespula) diabólica</i> .....		1	2			1			4
<i>Vespa (Vespula) maculifrons</i> .....			1	1	1				3
<i>Xylocopa virginica</i> .....	3		1						4
Total.....	3	3	9	2	1	2		2	22

Tabulations are also made of solitary bees other than those of the two genera *Halictus* and *Andrena* and for Hymenoptera other than the solitary bees (Table 6), but in these cases records by orchards and totals are given without records of temperatures.

#### PREVALENCE OF HONEYBEES

While honeybees were not collected, except in a few cases when mistaken for *Andrena* on high blossoms, some impression as to their prevalence in comparison with other insects was unavoidable. In orchard A, seven colonies of bees, maintained for pollination purposes, were located in or immediately adjacent to the orchard. For some

reason during the two days when collections were being made in this orchard, the honeybees from these colonies were working more in a cherry orchard a little over a quarter of a mile away than they were on the MacIntosh apple trees, which were just coming into full bloom. Later, after the collection of other insects was discontinued, the honeybees worked well on the apple blossoms. In orchard C a few weak colonies of bees had been placed; in orchard D there was one colony of bees; and near orchard F, almost directly across the road, was a small apiary, and honeybees were observed flying freely to and from these hives to the trees of the orchard. In the other orchards no provision had been made to supply honeybees, but a bee tree was discovered not far from orchard B, the colony appearing not to be strong.

The general impression was that in all the orchards honeybees outnumbered all other insects combined at least 3 to 1. In orchard G, noted for the scarcity of wild insects in 1931, the ratio was easily 100 to 1. Honeybees, it may be said, are more valuable in the orchards where these collections were made than all the wild insects together. This appears also to be true for the western New York fruit belt as a whole, based on more casual observations.

The honeybees worked earlier in the morning and later in the afternoon than did the wild bees, but bumblebee queens were often observed or captured at temperatures too low for honeybee flight. Bumblebee queens flew both early and late and were usually about the last insects to be seen each day. Honeybees were considerably more abundant in the middle of each day, at which time bumblebees appeared to be less prevalent.

#### THE POSSIBILITY OF INCREASING THE WILD SPECIES

It seems probable that the scarcity of wild insects on apple blossoms is due to a combination of factors incident to the agriculture of the fruit districts. The relatively high land values tend to reduce waste land and wood lots and also tend to eliminate the wide fence rows which are favored nesting places for some species. Cultivation reduces nesting and hibernating places, especially of solitary bees. Clean cultivation of orchards, where practiced, still further reduces the opportunities for the propagation of wild bees. It is possible that the efforts of fruit growers to control injurious insects in some degree serves to destroy individuals of those species which are beneficial. Beekeepers have observed that dusting destroys many honeybees, and it is probably equally disastrous to solitary bees.

The question naturally arises whether it would be feasible to make an effort to provide better conditions for wild insects. For the Hymenoptera this does not seem possible in any way that would make a practical appeal to fruit growers. For one species of fly (*Eristalis arbustorum*) already mentioned, there appears to be at least a possibility of increasing the number, as is suggested by the abundance of these insects from the roadside ditch near orchard B. These insects breed in polluted water which is not too deep, and which contains an abundant supply of food for the rat-tailed maggots constituting the larval stage of the species. It might be possible to increase the number of these insects by providing suitable breeding places far enough from residences as not to be offensive. A small preliminary experiment of

this kind was carried out during the summer of 1931, and toward the close of the season many puparia were in the adjacent soil. There are no results of value yet to be reported. Maggots of this species of fly are sometimes present by the millions in slow-flowing small streams or ditches polluted by cannery or milk wastes,<sup>4</sup> especially those with mud bottoms. Within the area of the fruit belt of western New York there are numerous canneries from which such waste might originate, but only one of the orchards (orchard D) from which collections were made was within flight range of such a cannery, and in this orchard *Eristalis* was not abundant.

### CONCLUSIONS

The sparsity of the population of wild insects in seven orchards in Monroe County, N.Y., where collections were made, is striking, but not surprising after some years of casual observations.

On the basis of the observations made, honeybees outnumbered all other insects.

There seems to be nothing that a fruit grower or group of fruit growers may do in a practical way to increase the population of wild insects, with the possible exception of certain syrphid flies.

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<sup>4</sup> CLAASSEN, P. W. BIOLOGICAL STUDIES OF POLLUTED AREAS IN THE GENESEE RIVER SYSTEM. In N.Y. State Conserv. Dept. Sup. to Ann. Rept. (1926) 16, A Biological Survey of the Genesee River System p. 38-46, illus. 1927.

# ECONOMIC STATUS OF THE GREEN STINKBUG WITH REFERENCE TO THE SUCCESSION OF ITS WILD HOSTS<sup>1</sup>

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## INTRODUCTION

There are frequent records of injury by the green stinkbug, *Acrosternum hilaris* Say, to many species of plants. Green bean pods, bolls of cotton,<sup>2</sup> the fruit of tomato,<sup>3</sup> and the fruit of both peaches<sup>4</sup> and grapes<sup>5</sup> are often attacked. Serious injury is generally local and somewhat periodic. Studies made in Virginia during the past seven seasons indicate that the insect has a distinct preference for certain native hosts and that the abundance of the insect depends on the presence of these plants. Injury to economic crops usually does not occur unless some of the wild plants fail to supply food. In a study of some of the causal factors involved in serious injury to Lima beans, the crop that has suffered most commonly in this state, the following conclusions have been reached: (1) Wild or uncultivated bushes and trees are naturally preferred; (2) a succession of hosts appears to be necessary for the insect to maintain itself in numbers; (3) cultivated crops are apparently damaged only when the wild-host succession is broken; and (4) severe injury to Lima beans is closely associated with a fungous disease organism.<sup>6</sup>

The studies on this pest were made in a farming section about 8 miles north of Richmond, Va. Clumps of trees occur here and there on the farms, and there are numerous tracts of timber. The section is partly bounded by the Chickahominy Swamp, which supports a dense thicket of native trees and bushes. A commercial nursery farm is located in this section. The fields are used for the cultivation of nursery stock and general farm crops, and there are a number of home gardens and home orchards. Practically all the native and cultivated plants of this section are found in this locality, and the bugs therefore had an opportunity to select the food preferred. During the period of time covered by these studies it was possible occasionally to make collections in other parts of the State and to visit farms where serious damage had been reported.

## HOST PREFERENCES

In Figure 1 some of the host plants are listed. The adult bugs are occasionally found on mulberry and blackberry in early summer. The other host plants are arranged in the order of apparent preference. The relative rank may vary somewhat for different seasons and

<sup>1</sup> Received for publication Dec. 23, 1932; issued June, 1933.

<sup>2</sup> SANDERSON, E. D. REPORT ON MISCELLANEOUS COTTON INSECTS IN TEXAS. U. S. Dept. Agr., Bur. Ent. Bul. 57:47-49, illus. 1908.

<sup>3</sup> MORRILL, A. W. PLANT-BUGS INJURIOUS TO COTTON BOLLS. U. S. Dept. Agr., Bur. Ent. Bul. 86:78-82, illus. 1910.

<sup>4</sup> WHITMARSH, R. D. THE GREEN SOLDIER BUG (*NEZARA HILARIS*). Ohio Agr. Expt. Sta. Bul. 310, p. 519-552, illus. 1917.

<sup>5</sup> STONER, D. THE SCUTELLEROIDEA OF IOWA. Iowa Univ. Studies Nat. Hist. 8(4):109. 1920.

<sup>6</sup> WINGARD, S. A. STUDIES ON THE PATHOGENICITY, MORPHOLOGY, AND CYTOLOGY OF NEMATOSPORA PHASOLI. Bul. Torrey Bot. Club 52:249-290, illus. 1925.

localities. While this insect feeds on a number of plants there are only a few preferred breeding hosts, and not all of these supply food during the main breeding period of adults and throughout the development period of the nymphs. Nearly all adults, eggs, and nymphs collected were on American elder (*Sambucus canadensis* L.), black or common locust (*Robinia pseudoacacia* L.), and honeylocust (*Gleditsia triacanthos* L.), three uncultivated hosts. Only two of these are seasonally well adapted to serve as hosts, namely, elder and honeylocust. Our studies indicate that the insect does not build up a large population when it is dependent on either of these plants. The former has the most regular fruiting habit, but farmers often destroy or cut this plant back to the ground; therefore, for one season at least, food is not available. However, sprouts generally come up and some berries are borne the first season, and an abundance of fruit the second year. Furthermore, where left undisturbed, other more hardy plants and vines tend to crowd out the elder in the course of a few seasons. The honeylocust is well adapted to serve as a host, but in 1929 and 1931

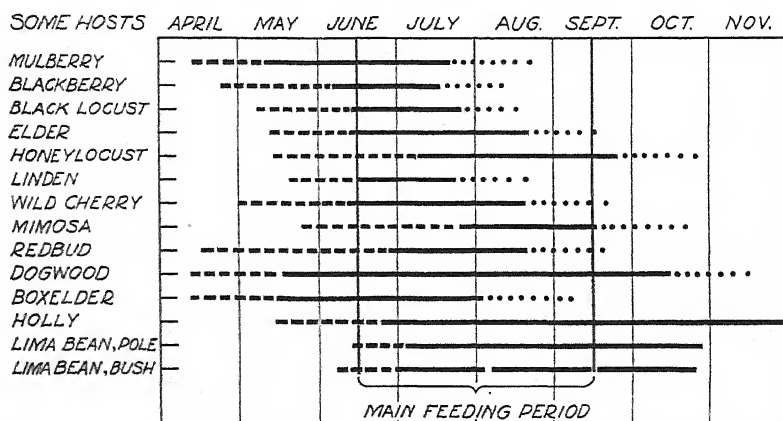


FIGURE 1.—Principal host plants of the green stinkbug and periods during which the seed pods and the fruit are in suitable condition for food as related to the main feeding period of the bug

it had no fruit in this locality. It appears that this tree frequently fails to bear fruit. Wild or black cherry (*Prunus serotina* Ehrh.) and flowering dogwood (*Cornus florida* L.) are natural breeding hosts with fruiting habits that fit in well with the feeding period of the insect, but heavy infestation has not been observed on either of these species. Many American holly trees (*Ilex opaca* Ait.) occur in this locality. The insect feeds on the berries, but it has been collected on holly only at rare intervals.

It may be noted from Figure 1 that several preferred breeding hosts have fruit suitable for food only a part of the season. For instance, black locust is a favorite host, but the pods ripen and dry by early August, about the time the earliest nymphs mature. The same is true of the American linden (*Tilia americana* L.) and American redbud (*Cercis canadensis* L.), both among the preferred hosts. The nymphs perish unless food is supplied the remainder of the period by other plants such as honeylocust, mimosa (*Acacia angustissima* (Mill.) Ktze., var. *hirta* (Nutt.)), dogwood, holly, grape (*Vitis* sp.), peaches (*Prunus persica* (L.) Stokes), or beans located

near enough for the nymphs to reach them by crawling. Hence, not many insects, as a rule, are found where such trees as black locust, redbud, or linden grow alone or together.

Lima beans (*Phaseolus lunatus macrocarpus* L.) are the preferred cultivated host, the pole varieties being more subject to attack than the bush types; however, in the trucking and canning sections where Lima beans are grown extensively, serious injury has not been observed. In most cases where bean fields were examined the insect was not found.

#### IMPORTANCE OF A SUCCESSION OF HOSTS

It appears that a sequence of wild plants in close proximity is necessary for this insect to maintain itself. Increase in numbers is impossible without such succession. One species of plants, though numerous, is not enough. Two, three, or four species growing relatively close together, or intermixed, appear to be essential. There are several reasons for this: (1) Nymphs are largely dependent on young or immature fruit or pods, and these are much preferred by the adults; (2) the main period during which nymphs are present continues about 12 weeks; and (3) the food supply is often cut short by unfavorable weather or other causes. Ideal conditions are approached where a succession of hosts exists to meet the essential needs of the adults, and to cover the development of all the nymphs. An excellent natural combination or succession appears to be black locust, honeylocust, and mimosa. This combination existed at the nursery in the section studied. Another succession favorable to the insect is found where either black locust, linden, or boxelder (*Acer negundo* L.) occur alone or together near grapes or growing green beans.

#### ATTACK ON ECONOMIC CROPS

Instances where farm or garden crops have been severely damaged so far as observed in Virginia, occurred where the host succession was incomplete or broken and the cultivated crop happened to be near and in condition to furnish the needed food. Usually the attack was made in August when nymphs migrated to the near-by crop from some wild host which had ceased to furnish food. Two instances of particularly severe attacks on Lima beans in the southern part of the State are given as examples. At Disputanta in 1928 practically all the beans in a home garden were ruined soon after the middle of August. Near by were four large linden trees. Food on lindens became exhausted, and the nymphs migrated to the Lima beans and grapes. In 1929 the Lima beans were planted a few hundred feet distant from the linden trees and were not attacked, although the trees were infested and a migration of nymphs occurred as in the previous season. Grapes and sweet peppers in the garden were severely attacked. There was a distinct migration each year. At Drewryville, Lima beans were ruined in one garden in 1929. Apparently this occurred because elder bushes, which grew in abundance on ditch banks and hedgerows, had been cut during the winter and the honeylocust trees on the farm bore no fruit pods in that season. The elder bushes grew and bore fruit in 1930, and the locust trees were full of pods. In mid-July the insects were very plentiful on these native plants, but none were found on the Lima beans although

they were planted in the same location as in 1929. The locality was visited again in early August, 1931. The bugs were found on elder bushes but none on beans.

Lima beans, in gardens at the nursery north of Richmond, even when planted close to natural host trees were not appreciably damaged, although adults and nymphs were very plentiful on the wild hosts each season. There was observed some tendency for adults to migrate to Lima beans during late July and early August, but this movement was too late for much injury, because the majority of the eggs had been deposited on native tree or bush hosts.

#### TRANSMISSION OF YEAST-SPOT DISEASE

When Lima beans are attacked, the injury is greatly increased by the presence of the bean yeast-spot organism, *Nematospora phaseoli*. In the two bean fields mentioned above, practically all the pods dropped off by the middle of August. This disease seems to be widespread in eastern Virginia and is generally associated with the feeding of this insect.

#### NATURAL ENEMIES

Very few natural enemies have been taken in Virginia except egg parasites. The average parasitism of eggs for six seasons at Richmond ranged from 16 to 27 per cent. In some localities as high as 55 per cent were parasitized. Six species of egg parasites have been reared and identified as follows: *Trissolcus euschisti* (Ashm.), *Anastatus reduvii* (How.), *A. pearsalli* (Ashm.), *A. mirabilis* (Walsh), *Telenomus dimmocki* (Ashm.), and *T. podisi* (Ashm.). The first two named were most numerous. Generally *T. euschisti* ranked first, and *A. reduvii* second. *A. pearsalli* was third in numbers but never numerous. The last three species were scarce.

Nymphs and adults are parasitized by tachinids. *Trichopoda pennipes* is the only species reared. Parasitism by tachinids has been very light except in one or two collections when eggs were very numerous on nymphs and adults. In one collection 65 per cent of the adults were parasitized.

#### SUMMARY

The green stinkbug, *Acrosternum hilaris* Say, which is a single-brood insect, feeds and deposits eggs by preference on wild hosts and requires green pods or fruit for food. The active breeding and development period is about three months which is generally too extended for one host to furnish a continuous supply of food. Studies made in Virginia for seven seasons indicate that a host succession is necessary for the insects to become numerous. Furthermore, it appears that cultivated crops are not severely attacked except when the host succession is incomplete or broken. When the insect feeds on Lima beans, the pods generally become inoculated with a fungous disease which may result in a complete crop failure, especially in humid seasons. Among natural enemies, egg parasites are most important. Six species of egg parasites have been reared and identified.



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## COMPARATIVE RESPONSES OF A SPRING AND A WINTER WHEAT TO DAY LENGTH AND TEMPERATURE<sup>1</sup>

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### INTRODUCTION

Garner and Allard (15)<sup>3</sup> have emphasized the fact that in certain cereals a fundamental physiological distinction between spring and winter varieties is shown by the difference in their response to the length of the daily period of illumination. Many other investigators (5, 8, 10, 11, 12, 19, 26, 37, 39) have observed such varietal differences, and their reports have called attention to the importance of the day-length response in the geographic distribution of the different varieties of cereals, as especially emphasized in the case of wheat by Wanser (39), Doroshenko (10, 11), and Tincker (37).

The present paper contains observations and measurements illustrating the differences in growth habit, rate of development, height, tillering, and yield of individual wheat plants of two varieties, Hard Federation, an extreme spring type, and Turkey, an extreme winter type, grown in the greenhouse under controlled day-length and temperature conditions.

### REVIEW OF LITERATURE

Reviews of the literature on photoperiodism in plants by Maximov (25), Kellerman (21), Redington (34), and Berkley (7) will serve to give the background of the present investigation.

As with most of the plants studied, the major emphasis in the case of wheat has been on the acceleration of reproductive processes by long light periods, and their retardation by short ones (2, 3, 4, 5, 9, 10, 11, 12, 15, 22, 23, 26, 36, 37). The extent to which the vegetative period can be shortened by increasing the day length differs for different varieties, the spring wheats having been found especially responsive to the hastening influence of a long light period. These investigations leave no doubt that the day-length responses of spring and winter types of cereals are as distinctive as their temperature adaptations (12, 16, 26, 29).

The length of the daily period of illumination has been found to determine not only height, tillering, and date of heading, but also root development (15, 26, 40), leaf and head development (10, 11), and

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<sup>2</sup> The writer is indebted to John W. Taylor, of the Division of Cereal Crops and Diseases, and to H. A. Allard, of the Division of Tobacco and Plant Nutrition, for their helpful suggestions in the course of this investigation; to A. G. Johnson and R. W. Leukel, of the Division of Cereal Crops and Diseases, for planning and supervising the installation of the temperature-control equipment; and to Fred Young for designing equipment used in controlling day length.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 886.

growth habit (19). In general it can be concluded from the literature that as the day length is made shorter the plants are more vegetative, with more tillers and larger leaves. As it is made longer, opposite effects are obtained. Some of Adams' results (1, 3) apparently constitute exceptions to these generalizations, but the explanation probably lies in his experimental procedure.

The interrelationship of temperature, light intensity, soil moisture, and other environmental factors in these various day-length effects has been discussed by many investigators (2, 4, 6, 12, 14, 15, 17, 18, 19, 26). Their reports confirm those of Garner and Allard in giving evidence that other factors may modify, to a certain extent, responses to the length of the daily light period.

#### METHODS

On December 2, 1930, seeds of a spring-wheat variety, Hard Federation (C. I.<sup>4</sup> No. 4733), and of a winter variety, Turkey (C. I. No. 1558), were sown in greenhouse benches at the Arlington Experiment Farm, Rosslyn, Va., in soil that was 10 inches deep. The plants were 4 inches apart in rows 6 inches apart. Different day-length conditions were provided for the different benches by means of artificial light and light-tight covers. The sowings were duplicated in the south-end sections of two adjacent greenhouses, one of which was kept at a favorable low temperature ( $12^{\circ} \pm 1^{\circ}$  C.), the other at a temperature too high for the best growth of wheat ( $21^{\circ} \pm 1^{\circ}$ ). The temperature was regulated by means of thermostatic control of the steam valves and of the dampers admitting cold air from the outside. An air distributor, 6 feet above the floor, circulated the incoming cold air fairly uniformly, so that the total variation in temperature in those parts of the houses used for the experiment was not over 1 degree.

Combination soil and air thermographs and standardized maximum and minimum thermometers were kept on stands 1 foot above the soil in each bench. During the period of temperature control—from December 2, 1930, the date of sowing, until April 3, 1931, when outdoor temperatures became too high—the cold house was held at  $12^{\circ} \pm 1^{\circ}$  C. except for a few hours on sunny afternoons, when it often rose to  $15^{\circ}$  in midafternoon, rarely to  $20^{\circ}$ . In the warm house the temperature was similarly well controlled at  $21^{\circ} \pm 1^{\circ}$  until April 8. On sunny afternoons it rose to  $25^{\circ}$ , but rarely above. Soil temperatures were approximately 2 degrees lower than the air temperatures in each house. Unless otherwise specified, the temperatures referred to in this paper are air temperatures.

Although the desired temperatures could not be maintained in either house after the first week of April, the low-temperature house was kept approximately 5 degrees colder than the other by means of a sprinkler system which kept a thin sheet of water running over practically the entire area of the glass of the roof and sides.<sup>5</sup>

<sup>4</sup> C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

<sup>5</sup> This method of lowering the temperature in the greenhouse, suggested by S. Karrer, was inexpensive and easily installed. Water pipes with spray nozzles at 1-foot intervals were laid on the roof just below the ventilators, and across the south end of the house. The nozzles were directed downward at such an angle as to cause the water to strike the glass a short distance from the pipe, where it spread to the width of the pane.

<sup>6</sup> Throughout the following discussions the terms "long-day," "short-day," "low-temperature," and "high-temperature" are used to denote the relative conditions under which the plants were grown, and not, in the sense of Garner and Allard (14), to denote the optimum condition for flowering. In the latter sense wheat is a long-day plant because flowering is induced by long days, and is delayed, as in Hard Federation, or prevented, as may occur in Turkey, by short days.

For the long-day<sup>6</sup> plants in each house the natural day was supplemented with light from six 100-watt Mazda C lamps in standard deep-bowl reflectors, distributed at 32-inch intervals, 4 feet above the bench. These lights were turned on and off automatically by a time switch which was adjusted periodically so as to maintain a constant 17-hour light period. The intensity of the artificial light at the level of the bench varied but slightly from 40 foot-candles. As the plants increased in height, the distance of the lights from the bench was increased to 5 feet. The thermograph records showed no discernible heating effects of these lights at the level of the plants.

The short-day condition was produced for the plants in another bench in each house by means of a light-tight black cloth cover supported on a slender framework 4 feet above the level of the bench. The cover was hung several inches out from the bench in order to insure ventilation. At the corners the side and end pieces overlapped and were attached to the framework with snap fasteners when the cover was in place. A piece of 1-inch pipe constituted the back edge of the framework, and to it was attached a crank handle by means of which the curtain could be rolled upon the pipe and lifted to one side when not in place over the bench. The plants were darkened every day from 4 p. m. until 8 a. m., leaving them a light period of eight hours in the middle of the day. The cover was thick enough to protect the plants from the lights of the neighboring long-day bench, and was always in place before these lights were turned on.

Control plants with the natural light period were grown on an adjacent bench and protected from the lights over the long-day bench by a heavy black curtain 6 feet high, hung in the aisle and extending well past the ends of the benches. This curtain was in place whenever the lights were on, and was pulled to one end, where it did not shade the plants, when the lights were turned off. The natural length of day, from sunrise to sunset, increased over the period of the experiments from 9.5 hours in December to 15 hours in June.

The following observations and measurements are based on approximately 60 plants of each variety, exclusive of border plants, grown under each of these six different conditions of day length and temperature.

## RESULTS

### EFFECTS OF DAY LENGTH AND TEMPERATURE ON RATE AND TYPE OF DEVELOPMENT

The seedlings of the low-temperature house ( $12^{\circ} \pm 1^{\circ}$  C.) emerged in 10 days, and those of the high-temperature house ( $21^{\circ} \pm 1^{\circ}$ ) in 6 days. The long (17-hour) light period so accelerated the development of the young plants of the spring variety, Hard Federation, that at first they greatly exceeded the others in height. At the natural and at the short (8-hour) days, Turkey, the winter variety, assumed, for about three months, the recumbent growth habit, typical of young plants of this and similar varieties in winter; but at the 17-hour day the plants were erect or semierect from the first, as

in experiments previously reported by the writer (19).<sup>7</sup> In the low-temperature house these erect plants matured normally and produced good yields of grain. In the high-temperature house they remained largely vegetative, as did the Turkey plants of the other light periods at this temperature.

The contrast between the erect growth of the young plants of Turkey at the long day and their recumbent growth at the shorter ones, especially marked at the low temperature, is illustrated in Figure 1, which also shows the relative development of Hard Federation plants of the same age. The plants of Turkey with the 8-hour day, A, were still prostrate at the time the photographs were taken, while the control plants, B, with the natural day, which was nearly 11 hours long at this time, had become semierect, indicating the resumption of active growth. The 17-hour-day plants, C, were never prostrate.

The comparative rates of development of Hard Federation and Turkey under the different conditions are shown in Table 1, where the dates of first flowering, together with the averaged growth measurements and their probable errors, are recorded. Heading occurred during the week or 10 days preceding the date of first flowering, except in the case of the short-day plants, which headed irregularly over a longer period.

Representative plants from the different environments, selected for photographing when maximum growth had been attained but while most of the foliage was still green, are shown on the same scale in Figures 2 and 3. Later, after the plants had matured and their measurements were obtained and averaged, the photographs of plants conforming most closely to the averages for the groups were chosen for the illustrations. Occasionally none of the plants photographed from a given group happened to conform to the size shown by the averages in Table 1 to be typical. Such was the case in Figure 3, where all the plants have more than the average number of tillers for their group.

<sup>7</sup> Since the present paper was prepared, Forster, Tincker, Vasey, and Wadham (13) have reported that in their wheats growth did not completely cease at temperatures as high as 10° C., and that therefore they do not agree with the writer's statement (19, p. 119) that a resting stage is produced by 8 and 9.5-hour light periods at this temperature. The term "resting stage" was used in this earlier publication to designate the prostrate stage of development of Turkey in winter, preceding the erect leafy growth of the later period of the tillering stage. In Turkey and similar varieties the plants are recumbent during this early tillering stage, as described and illustrated by Percival (31, p. 69-71), and growth is almost imperceptible over a considerable period. A rest period is referred to by Maximov (28, p. 290) and others as typical of some cereals in winter, "... \* \* \* when growth completely ceases and the plant enters into a state of rest." Maximov states further: "This condition may be of various intensity and duration. It may involve the whole plant or only parts of it." Undoubtedly, as Forster and his associates have stated, all growth during the recumbent stage is not completely stopped in winter cereals at a temperature as high as 10° to 12°, the low temperature of this and the previous investigation (19). However, whether or not there is a true dormant or rest period is immaterial to the purpose of these investigations, the term being used with the sole purpose of designating the period during which the plant is in the prostrate or recumbent condition characteristic of the early tillering stage of Turkey and similar winter wheats grown with short light periods.

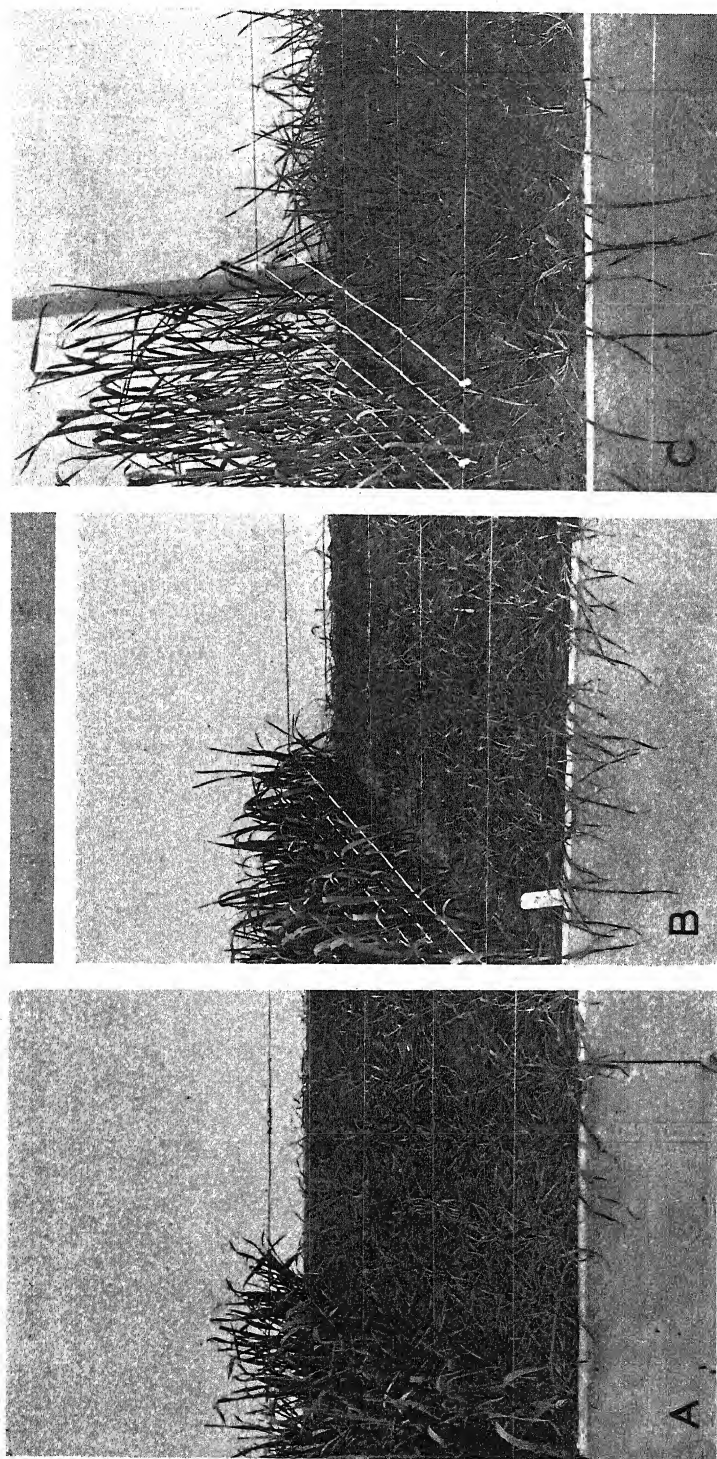


FIGURE 1.—Comparative development of winter-sown Turkey wheat (at right in each photograph) and Hard Federation (at left) 10 weeks after sowing, grown with constant low temperature ( $12^{\circ}\pm 1^{\circ}$  C.) and different light periods: A, Short day (8 hours); B, natural day (9.5 to 10.5 hours); C, long day (17 hours)

TABLE 1.—*Effects of day length and temperature on the average height, tillering, head development, and yield of a spring wheat (Hard Federation) and a winter wheat (Turkey) sown December 2, 1930*

Item	Hard Federation at 12°±1° C.		
	Short day (8 hours)	Natural day (9.5-15 hours)	Long day (17 hours)
Date of first flowering.....	May 9	Apr. 13	Feb. 19
Dry weight of straw.....grams	9.5	7.7	1.2
Height of plants.....centimeters	90±0.75	98±0.93	66±0.86
Total tillers per plant.....number	5.8±.27	3.7±.16	<sup>a</sup> 3.8±.16
Fertile heads per plant.....do	3.9±.19	3.5±.17	1.3±.10
Length of heads.....centimeters	9.5±.07	8.6±.06	5.4±.05
Kernel weight.....gram	.035±.0007	.054±.0005	.055±.0009
Weight of grain per fertile head.....do	.51±.02	.85±.06	.37±.02
Yield of grain per plant.....do	1.91±.11	2.81±.21	.45±.05
Hard Federation at 21°±1° C.			
Date of first flowering.....	Apr. 25	Mar. 18	Jan. 13
Dry weight of straw.....grams	10.3	8.1	<sup>(b)</sup>
Height of plants.....centimeters	82±1.20	84±1.07	48±.77
Total tillers per plant.....number	8.1±.32	6.2±.28	2.7±.11
Fertile heads per plant.....do	2.9±.14	4.1±.25	1.6±.06
Length of heads.....centimeters	8.5±.08	7.8±.05	5.4±.06
Kernel weight.....gram	.028±.0004	.039±.0007	.036±.0007
Weight of grain per fertile head.....do	.24±.04	.61±.03	.38±.02
Yield of grain per plant.....do	.77±.06	2.40±.18	.67±.03
Turkey at 12°±1° C.			
Date of first flowering.....	June 8	May 16	Apr. 13
Dry weight of straw.....grams	12.9	8.7	3.8
Height of plants.....centimeters	110±0.76	115±0.88	101±0.71
Total tillers per plant.....number	6.6±.28	5.3±.24	3.4±.09
Fertile heads per plant.....do	2.0±.13	4.3±.17	2.9±.09
Length of heads.....centimeters	13.7±.11	10.0±.05	8.4±.04
Kernel weight.....gram	.022±.0008	.035±.0002	.043±.0005
Weight of grain per fertile head.....do	.23±.02	.98±.04	1.15±.03
Yield of grain per plant.....do	.53±.04	4.06±.19	3.57±.09

<sup>a</sup> Very short secondary tillers without grain and but a few inches long, characteristic of these plants, were not included in this average. If included, the average would be 6.9.

<sup>b</sup> Straw inadvertently discarded.

<sup>c</sup> In the year preceding and the year following, in both of which the seed was sown 2 weeks later than in this experiment, the heads of Turkey at the short day produced no grain. (Table 2.)

Hard Federation was extremely sensitive to the forcing action of the lengthened light period. At both temperatures the plants with the 17-hour day were forced into the reproductive stage so rapidly that there was insufficient leaf development before maturation processes began. The plants were extremely stunted, with short, narrow leaves and small, poorly developed heads. (Fig. 2, C, F.) In the low-temperature house they began to flower the third week of February, 10 weeks after emergence. They were eight weeks ahead of the control plants (fig. 2, B), which flowered the second week of April. In the high-temperature house, development was still more rapid, the long-day plants flowering the second week of January, only five weeks from emergence and nine weeks ahead of the control plants (fig. 2, E), which bloomed the third week of March.

Maturation of the short-day plants of Hard Federation was as markedly retarded as that of the long-day plants was hastened. Whereas the rapid development at the 17-hour day had resulted in stunted plants with reduced foliage, the 8-hour day caused a highly vegetative type of growth, with increased tillering and large leaves. (Fig. 2, A, D.) In the cold house the plants began to flower the second week of May, a month later than the control plants with the natural light period. At the higher temperature they flowered the last week of April, more than five weeks later than the natural-day plants in that house.

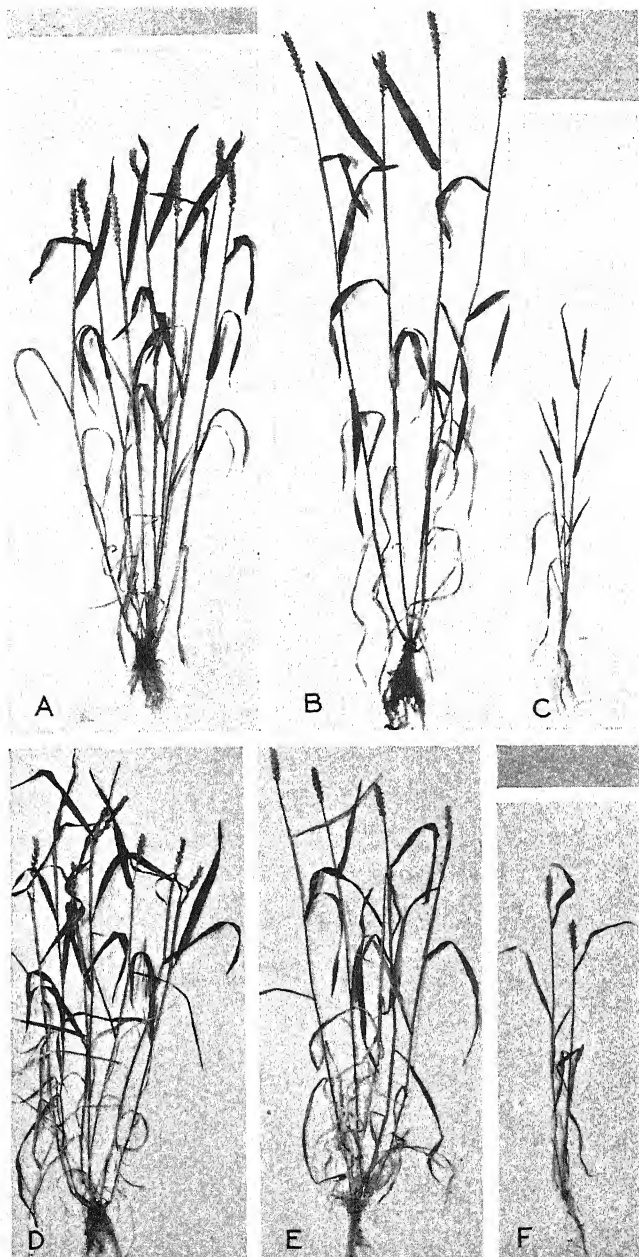


FIGURE 2.—Mature plants of winter-sown Hard Federation wheat grown with different light periods and temperatures ( $12\pm 1^{\circ}$  and  $21\pm 1^{\circ}$  C. for the first four months): A, B, C, Short-day, natural-day, and long-day plants, respectively, grown at the lower temperature; D, E, F, short-day, natural-day, and long-day plants, respectively, grown at the higher temperature



At the low temperature, Turkey, the winter variety, while less susceptible to the forcing action of the lengthened light period than Hard Federation, also developed more rapidly with the 17-hour day than with the natural day. The plants were not stunted as were the

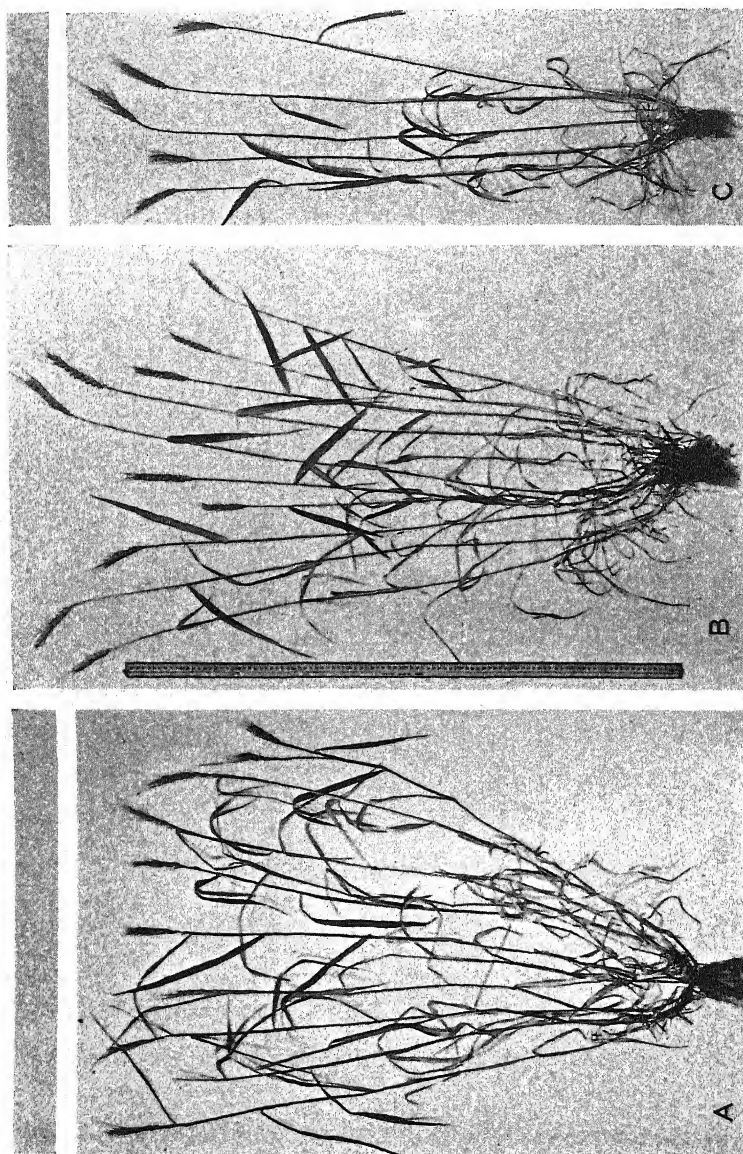


FIGURE 3.—Mature plants of winter-sown Turkey wheat grown with different light periods and, for the first four months, at a constant temperature of  $12\pm1^{\circ}\text{C}$ .: A, Short day (8 hours); B, natural day (9.5 to 15 hours); C, long day (17 hours)

long-day plants of the more rapidly developing spring variety, but on the contrary compared favorably, except for their reduced tillering, with the natural-day controls. (Fig. 3, B, C.) Flowering occurred during the second week of April, five weeks ahead of the natural-day plants, while those of Hard Federation growing beside them had



flowered the third week of February, eight weeks ahead of their controls. The short day resulted in a vegetative type of growth, as in Hard Federation, with maturation considerably delayed. (Fig. 3, A.) Thus, at the 8-hour day Turkey did not head until June 8, three weeks later than the natural-day plants.

In the high-temperature house but few heads were produced by Turkey at any day length, most of the plants being completely sterile. They remained green and mostly vegetative until the following October, when they were discarded. Excessive tillering, together with the common failure of the culms to elongate, gave the plants of all the light periods the bushy appearance characteristic of spring-sown winter wheats that fail to head (6). The photographs in Figure 4 were taken in August, when the plants were still green, long after all the other lots had ripened. The vegetative condition of the long-day plants in this house gave evidence of the fact that the ordinarily dominant action of the light period becomes imperceptible at temperatures unfavorable for reproduction.

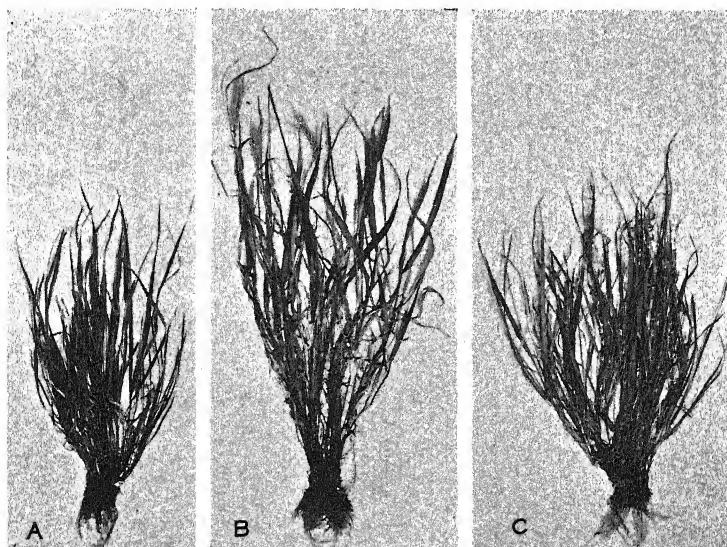


FIGURE 4.—Effect of the higher temperature ( $21^{\circ}\pm 1^{\circ}$  C. for first four months) on the growth of Turkey at the different day lengths: A, Short day (8 hours); B, natural day (9.5 to 15 hours); C, long day (17 hours)

The tendency of the shortened day to favor continued vegetative growth and that of the long day to inhibit it are evident in both the winter and the spring variety on comparing the weights of the dried plants from the different environments. The average weights of straw per plant shown in Table 1 were obtained by weighing the fully ripened air-dry plants, exclusive of the heads and roots. In the low-temperature house the average weight of a short-day plant of Turkey was more than three times that of a long-day plant. In the case of corresponding plants of Hard Federation, the short-day plants averaged eight times the weight of those with the long day. The straw of the long-day plants of Hard Federation from the high-temperature house was inadvertently discarded, but approximately the same relation is evident from the appearance of the plants in

Figure 2, F. The weights of the natural-day plants of both varieties were intermediate between those of the short and long days, but re-

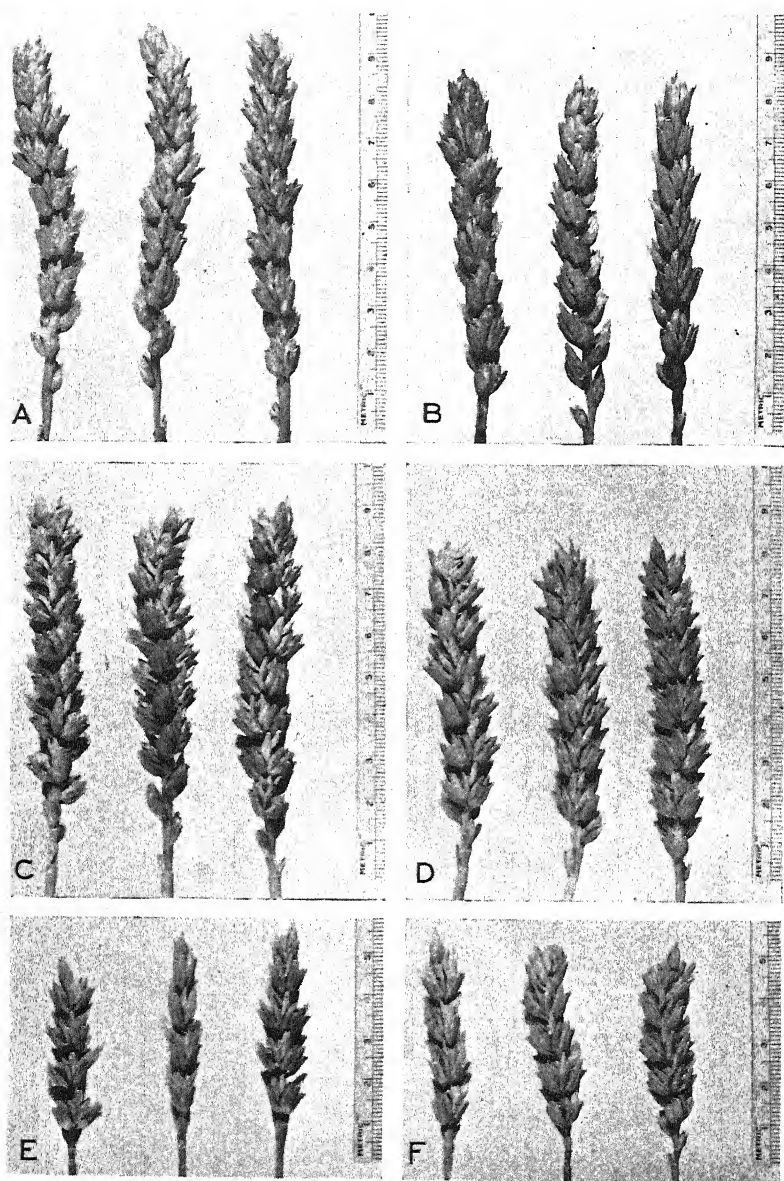


FIGURE 5.—Typical heads of Hard Federation wheat grown with different day lengths and temperatures ( $12^{\circ}$  and  $21^{\circ}$  C. for the first four months): A and B, Short day (8 hours) at the low and high temperatures, respectively; C and D, natural day (9.5 to 15 hours), at the low and high temperatures, respectively; E and F, long day (17 hours) at the low and high temperatures, respectively

duction in size by the long day was always more pronounced than was the increase brought about by the short day.

The average heights recorded in Table 1 represent the final measurements on the mature plants, including the heads. The data show that the 17-hour light period shortened the plants in every case, the effect on Hard Federation being much more extreme than the effect on Turkey. The 8-hour day also reduced the average height of the plants of both varieties, but the effect was less pronounced than that of the lengthened light period. At every day length the plants of the cold house were taller than the corresponding plants of the warm house, the difference reflecting the injurious effects of temperatures above 20° C. on the growth of even the spring wheat.

The number of tillers per plant was highest in both varieties at the short day, and generally lowest at the long day. If only normal tillers with heads were counted, the number was always lowest at the long day. At equivalent day lengths, more tillers were produced at the high than at the low temperature, except in Hard Federation at the long day.

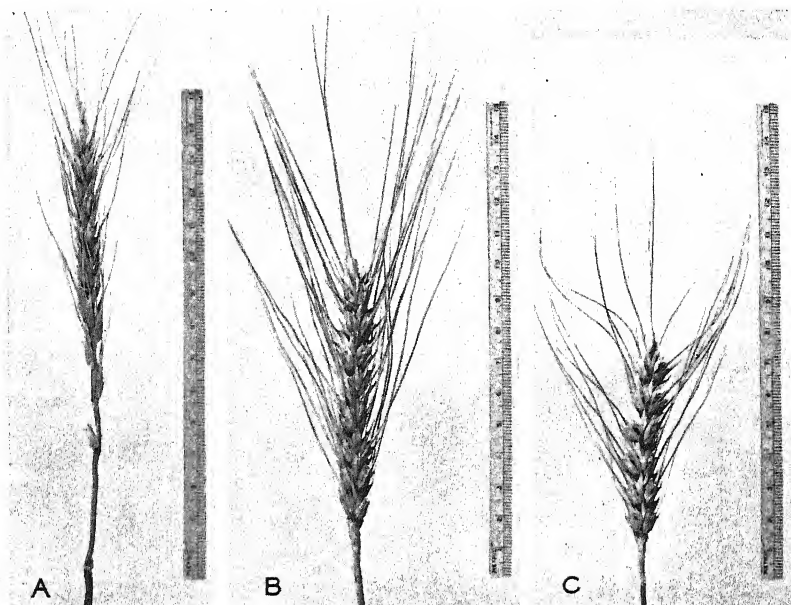


FIGURE 6.—Typical heads of Turkey grown at  $12^{\circ}\pm 1^{\circ}$  C. for the first four months, with different light periods: A, Short day (8 hours); B, natural day (9.5 to 15 hours); C, long day (17 hours)

Among the conspicuous formative effects of the length of the light period were the effects on head length. (Table 1.) In both Hard Federation (fig. 5) and Turkey (fig. 6) the long day produced abnormally short heads. The short day produced very long heads, largely as the result of abnormal elongation of the basal internodes of the rachis. This elongation was especially pronounced in Turkey, some of the heads reaching 20 cm, measured from the lowest node of the rachis. Occasional abnormal heads like those in Figure 7, showing indeterminate growth of the spikelets, were produced by Turkey at this day length.

Other structural abnormalities were the shortened peduncles of the plants of the short day. (Figs. 2, A, D; 3, A.) Sometimes elongation of the peduncle ceased as soon as the head emerged from the boot.

Relative kernel size for the different groups of plants is illustrated in Figure 9. This figure shows the reduced size of the grains of the short-day plants of both varieties, and the large plump grains of the long-day low-temperature plants. The high-temperature grains were always smaller than the corresponding ones from the low-temperature plants.

The following year (1932) grain from each of these seed lots was grown in the low-temperature house with the natural light period.

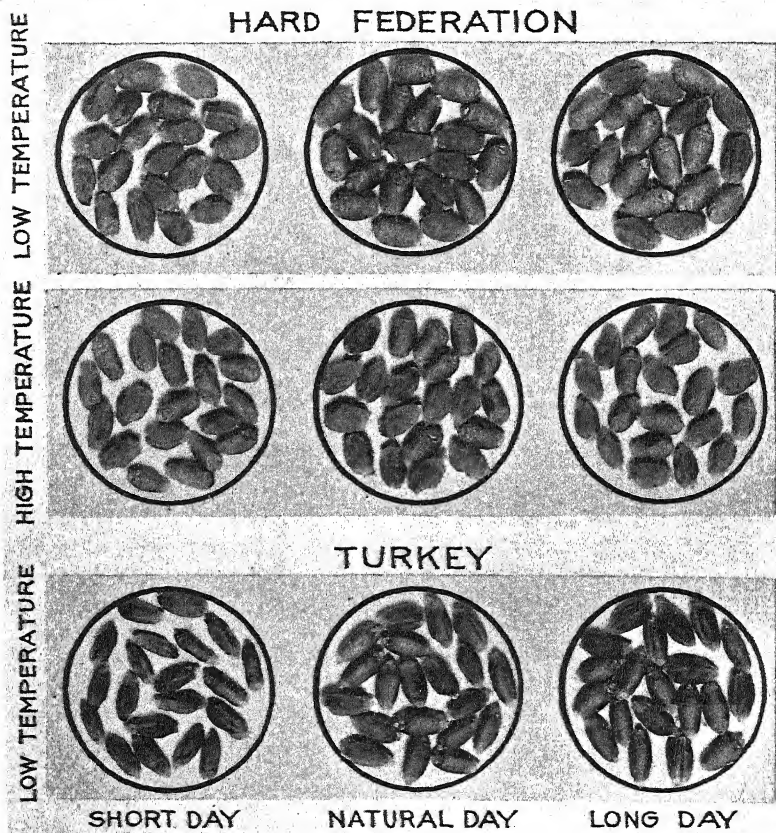


FIGURE 9.—Effects of day length and temperature on kernel development of wheat. (Twenty grains in each circle)

Except for somewhat reduced size in early growth stages, the plants grown from the small shriveled grains of the short day were very similar to those from the larger grains of the other day lengths. There were no significant differences in height, tillering, dry weight, or yield of any of the plants. It is concluded, therefore, that the conditions of day length and temperature under which the grain was produced had no appreciable effect on the development of the plants grown from the grain.

## SUPPLEMENTARY EXPERIMENTS

The question arises in connection with the generalizations discussed in the preceding sections as to the reproducibility of these effects of day length and temperature. Data from several similar experiments but with different dates of sowing and different spacings are summarized in Table 2 in order to show that the relative effects of the different environments and the varietal differences in the responses to these environments were not fortuitous variations.

The experiment with spring-sown grain (A) included only Hard Federation, and differed from that just described in its closer spacing, the plants being 2 instead of 4 inches apart. The experiment with grain sown in the winter of 1931 (B) also differed with respect to spacing, in that the rows were 1 foot apart instead of 6 inches. The experiment with grain sown in the winter of 1929 (C) resembled that described in the preceding sections of this paper, the plants being spaced at 4-inch intervals in rows 6 inches apart. The experiment A differed from all the rest in that temperature control could be maintained for the first month of growth only, whereas the winter sowings had controlled temperatures for the first four months. The temperatures were similar in all the experiments, namely, between 10° and 14° C. in the low-temperature house and between 20° and 23° in the high-temperature house. All border plants were discarded.

The data in Table 2 are in accordance with the generalizations indicated by the data in Table 1. They show that the 17-hour day accelerated the development of both Hard Federation and Turkey and produced smaller plants, with lower dry weights and reduced tillering. Acceleration of heading was less marked in Turkey than in Hard Federation, as before. Yield per head at the long day was always higher than at the natural day in Turkey but lower in Hard Federation. The short day consistently produced large vegetative plants with the most tillers, whose development was retarded. Grain production was decreased or, in Turkey, entirely prevented by the short day.

A significant difference between the Turkey plants of the experiments of Table 2 and those of Table 1 was the failure of the former to flower at the short day. The difference may be attributed to the fact that the grain for the experiments of Table 2 was sown two weeks later than that of Table 1, the retardation by the short day resulting in the later-sown lots reaching the preflowering stage when greenhouse temperatures were higher.

TABLE 2.—Effects of day length and temperature on average height, tillering, head development, and yield of Hard Federation and Turkey wheat in supplementary experiments, 1930–1932

Item	Experiment <sup>a</sup>	Hard Federation						Turkey		
		Low temperature			High temperature			Low temperature		
		Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)	Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)	Short day (8 hours)	Natural day (9.5–15 hours)	Long day (17 hours)
Date of first flowering	{ A B	June 13 May 4	May 15 Apr. 17	May 7 Feb. 25	June 30	May 4	Apr. 18		May 15	May 4
Dry weight of straw	{ A B	2.9 6.3	2.7 8.2	1.1 1.6	3.8	2.9	0.8	12.7	11.4	8.1
Height of plants	{ A B	72 76	81 99	55 64	72	62	37	76	118	119
Total tillers per plant	{ A B	1.8 5.9	1.3 5.0	1.1 3.9	3.9	1.5	1.4	12.8	9.3	7.2
Fertile heads per plant	{ A B	1.1 4.4	1.1 4.4	1.0 1.1	1.5	1.4	1.0	0	8.6	7.0
Length of heads	{ A B	9.0 8.9	7.2 8.1	4.6 4.6	7.8 8.0	7.2 7.6	4.3 3.9			
Kernel weight	{ A B C	.030 .021 .034	.045 .049 .049	.045 .048 .045	.03	.041 .041	.033	0 0	.032 .038	.037 .043
Weight of grain per fertile head	{ A B C	.23 .26 .79	1.00 1.44 1.17	.51 .33 .03	.11 .40	.95 .88	.30 .21	0 0	.78 1.04	.88 1.18
Yield of grain per plant	{ A B	.25 1.08	1.10 6.23	.51 .38	.16	1.33	31	0	6.89	6.07

<sup>a</sup> Experiment A, seed sown Mar. 2, 1931; B, seed sown Dec. 15, 1931; C, seed sown Dec. 13, 1932.

## DISCUSSION

A shortened daily light period induces an abnormally vegetative type of growth in wheat, whereas, at temperatures low enough to permit heading, a lengthened one induces abnormally hastened reproduction. However, the results of the present investigation show that plants so rapidly forced into the reproductive stage by a long light period are not made more fruitful thereby, but, on the contrary, yield is reduced, especially in the case of a spring wheat like Hard Federation. Plants of this variety are stunted, the leaves small, and the heads often sterile. The winter variety, Turkey, is not forced so rapidly into reproduction by a long day, but, at favorably low temperatures, it grows tall and vigorously, its leaves are large, and it produces an even higher yield of grain per head than it does with the natural day. Nevertheless it does not produce the greatest yield per plant at the long day, for the number of heads is reduced.

It is interesting to note that although a shortened light period always increased the number of tillers per plant in both varieties, and, in Turkey, a lengthened one increased the kernel and head weights, still the highest total yield of both varieties was produced by the plants with the natural day, possibly because of the favorable effect of the changing light period (19, 27, 33), which increased from 9.5 to 15 hours during the experiments.

Differences between the reactions of the spring and the winter variety to the different environments were conspicuous at every stage of development. In the early tillering stage, the prostrate growth habit of the short-day and natural-day plants of Turkey distinguished them from the erect plants of Hard Federation in the same benches. Within the first 5 to 10 weeks after emergence, depending on the temperature, heads were appearing in the long-day plants of Hard Federation, while Turkey in the same bench was still in the tillering stage. Turkey continued in this vegetative stage for some time after the spring plants had fully matured; so, although the long-day plants of Hard Federation were much taller at first than the adjacent plants of the winter variety, their growth was soon ended by the early maturation of the heads, leaving the plants abnormally short and with reduced leaf and head development. At the low temperature the more slowly developing long-day plants of Turkey grew to twice the height of the Hard Federation plants beside them and produced fully developed leaves and heads. The extreme reduction in yield in Hard Federation at the long day, with less injury at the short day, and, conversely, in Turkey, the occurrence of extreme injury at the short day with increased head and kernel weights at the long day, gave interesting evidence of a fundamental distinction between spring and winter wheats. Other evidence of the physiological difference between the two varieties was, of course, the greater tolerance of Hard Federation for the higher temperatures.

The appearance and measurements of the plants in the present investigation show that excellent growth can be obtained in the greenhouse with no source of ultraviolet light. In 1931 the control plants of the winter variety, Turkey, with the natural length of day averaged 115 cm in height at maturity, with an average of 5.3 tillers and 4 g of grain per plant, testing 62 pounds to the bushel. In 1932, when the rows were 1 foot apart instead of 6 inches, still larger plants



were obtained which averaged 8.6 fertile heads, giving 6.9 g of grain per plant. (Table 2.) The plants, therefore, compared favorably with field-grown plants (6) of this variety.

The excellent growth and yield of the plants of Turkey at the 17-hour day were of special interest because the recumbent growth habit typical of this variety in a natural winter environment at this latitude was inhibited by the long light period. The subsequent normal development of the plants showed, in agreement with the conclusions of other investigators (29, 30, 38), that a resting or recumbent stage was not essential for good yield. Since 17-hour-day plants of this variety yielded so well in all the experiments, the suggestion that the failure of spring plantings of winter wheat to head may be attributed to too long light periods (29)—or to the resulting absence of a prostrate growth phase in the tillering stage (19)—now seems untenable, for the longest summer day is only 15 hours at this latitude. In view of the fact that temperatures above 20° C. in the greenhouse are so deleterious to winter wheat (20) and, in the present experiments, always prevented normal development at all the day lengths used, it seems probable that the onset of warm weather before the end of the long vegetative period is responsible for the abnormal growth of Turkey in spring sowings.

Gassner (16), McKinney and Sando (24), Maximov (26), and others have shown that stimulation of the transition from vegetative to reproductive growth in winter wheat can be brought about by germinating the plants at temperatures near freezing. However, Murinov (30) and Wacar (38) have reported that, in the winter varieties they studied, very low germination temperatures were not essential for heading. It now seems from other investigations (29, 32) that very low temperatures in early stages stimulate heading in the case of late sowings only, for when germination takes place in the winter such low temperatures have no noticeable effect, presumably because the short winter day supplies a similar stimulus (27). In accordance with these reports, the low-temperature plants of the present investigation (for all of which the seed was sown in December) progressed normally from the vegetative condition to reproduction with normal yields of grain without experiencing either soil or air temperatures below 9.5° C. It would seem, therefore, that statements (35, p. 53) to the effect that winter cereals "grown from seed germinated above 5° C. and not allowed during growth to fall below this temperature do not form ears but grow vegetatively only" are not applicable to the winter wheat used in these investigations.

It is of interest to note that three groups of the Hard Federation plants, including one in the low-temperature house (Table 1) flowered within the period of temperature control, which continued through the first week of April. With the exception of the natural-day plants, for which the light period was increasing, these plants flowered with no apparent change in the external environment other than the change in light intensity as the season advanced. This fact would appear to controvert the opinion (27) that a rise in temperature as well as an increase in day length is necessary for further development after the plant has reached the preflowering stage.

Wanser (39) thought that specific critical day lengths are necessary to bring about the transition from the vegetative to the reproductive



phase of growth in winter wheats, and to initiate jointing and heading. The fact that in the present investigation Turkey plants have progressed from the seedling stage to culm elongation, flowering, and maturation at both the constant short and constant long days shows that the stimulus of a changing length of day is not essential for development in all winter wheats.

Turkey and some other varieties of winter wheats have been shown (19, 27, 33) to be stimulated to more rapid development by transferring them from short to long light periods in early stages of growth. In the writer's experiments, transplanting young Turkey plants from the short-day to the long-day bench at the age of 2 months, when they were still in the recumbent stage, resulted in rapid emergence from this stage. Culm elongation proceeded so rapidly that the plants soon surpassed all the others. Flowering and maturation of such transplanted plants in experiments of three different years occurred without exception from one to two weeks earlier than in the adjacent plants with the constant long day, which were themselves two months earlier in heading than were the natural-day plants.

The various reports (16, 19, 24, 26, 27, 29, 32, 33, 38) of favorable effects of a change from low temperatures or short days in early growth stages to higher temperatures or to longer days in later ones have proved that under some conditions such changes hasten the subsequent transition from vegetative to reproductive growth. There still remains the question as to what brings about the transitions in growth phases that occur in the absence of any apparent change in the external environment.

#### SUMMARY

Hard Federation, a typical spring wheat, and Turkey, an extreme winter type, were grown in the greenhouse with short (8 hours), natural (9.5 to 15 hours), and long (17 hours) light periods at each of two temperatures, a low one ( $12^{\circ} \pm 1^{\circ}$  C.), favorable for the growth of wheat, and one too high for the best development of even the spring variety ( $21^{\circ} \pm 1^{\circ}$ ). Temperature control was maintained from the first of December until early April.

The long day hastened the development of both varieties, but the acceleration was much more pronounced in the case of the spring variety. The short day retarded the development of both varieties.

At the low temperature, the short day increased leaf size, head length, and generally the number of tillers, and decreased kernel weight and yield of the plants of both varieties, causing complete sterility in Turkey in two out of three experiments. The long day decreased dry weight, tillering, head length, and total yield of grain in both varieties, although Turkey's yield per head and average kernel weight were always highest at this day length. Yield per plant of this variety at the long day was reduced because of the reduction in tillering.

The short day was more damaging than the long day to the yield of grain in Turkey, whereas the long day was the more damaging to Hard Federation.

The high temperature was more damaging to the winter variety than it was to the spring variety, heading of the former being sparse and the growth of the plants abnormal above  $20^{\circ}$  C.

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# EFFECTS OF CHLORINE, BROMINE, AND FLUORINE ON THE TOBACCO PLANT<sup>1</sup>

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## INTRODUCTION

A considerable body of data is available on the influence of the element chlorine, applied as chlorides, upon the growth of crop plants. No special investigation, however, appears to have been made of the influence of the closely related elements, bromine and fluorine. The object of much of the work with chlorine has been to determine whether chlorides are beneficial or harmful to the crop. The literature on this subject, as reviewed by Tottingham (10)<sup>3</sup> and by Lomanitz (6), shows that it has long been the practice among growers to apply sodium chloride as a fertilizer material and that the effect of chlorides depends partly on such factors as the quantity of chlorine applied; the kind of crop; the type, reaction, and moisture content of the soil; and the meteorological conditions prevailing during the growing season.

The influence of chlorine on tobacco has received careful study by Garner and his associates (4). In comparative tests with sulphate of potash and chloride of potash on Durham sandy loam, these investigators found an average increase of 10 per cent in yield on plots to which chlorides were applied. Two reasons are given for the stimulatory effect of chlorides: (1) Chlorine increases the solubility of magnesium, enabling the plant to absorb enough of this element to meet its nutritional requirements; and (2) the presence of chlorides within the tobacco plant enables it, by maintaining an increased water content, to better withstand periods of drought. Garner and his associates noted, moreover, that plants supplied with an excess of chlorine were dwarfed and that their leaves were glossy, extremely thick, and brittle. In extreme cases the margins of the leaves were curled upward; such leaves were found to have an abnormally high water content and to be gorged with starch. These workers also found that an excess of chlorine lowers the market value of the cured leaf through its deleterious effects on color, elasticity, and keeping quality. The manufactured product may be affected also, since tobacco of excessive chlorine content is high in moisture and low in organic acids and hence does not burn readily.

Since, so far as is known, no one has investigated the possible beneficial or harmful effects of the elements bromine and fluorine upon the tobacco plant and since no detailed study seems to have been made of the effects of chlorine, bromine, or fluorine upon the anatomy of the leaves or upon the physicochemical properties of the sap, an investigation of these matters was undertaken and is reported herein.

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<sup>2</sup> The writer wishes to acknowledge the cooperation of Frederick A. Wolf, Duke University, Durham, N. C., in this investigation.

Reference is made by number (*italic*) to Literature Cited, p. 899.

## MATERIAL AND METHODS

Material was secured both from tobacco plants (*Nicotiana tabacum* L.) grown in the field and from plants grown under glass. The field-grown material was collected in two localities, one about 14 miles north of Durham, N. C., and the other about 16 miles south of Durham. The plants in the greenhouse were grown in 6-inch porous pots in a soil consisting of a mixture of loam and river-washed sand. A small quantity of 8-4-4 fertilizer was stirred into the soil before the plants were transplanted. The soil was kept moist by applications of dilute solutions of salts interspersed with applications of tap water. Each lot of plants, except the controls, was given applications of one of the following salts: Ammonium chloride ( $\text{NH}_4\text{Cl}$ ), magnesium chloride ( $\text{MgCl}_2$ ), potassium chloride ( $\text{KCl}$ ), sodium chloride ( $\text{NaCl}$ ), ammonium bromide ( $\text{NH}_4\text{Br}$ ), sodium bromide ( $\text{NaBr}$ ), potassium bromide ( $\text{KBr}$ ), ammonium fluoride ( $\text{NH}_4\text{F}$ ), potassium fluoride ( $\text{KF}$ ), or sodium fluoride ( $\text{NaF}$ ). Saucers placed beneath the pots prevented loss of salts by drainage and permitted the salts to accumulate in increasing concentrations. When incipient collapse indicated that the plants could tolerate no more salts, suitable moisture content was maintained by tap water alone. A canopy of cheesecloth was used to provide partial shade.

Material for anatomical study was cut from the leaves by means of a cork borer and was fixed in formalin-acetic-alcohol solution. Paraffin sections were cut  $20\mu$  to  $30\mu$  in thickness and were stained with Heidenhain's iron-alum haematoxylin.

Material for physicochemical studies was collected from the field between 8 and 9 a. m., and from the greenhouse at about 7.30 a. m. Each sample from the field consisted of representative leaves taken from a dozen or more plants. The samples were immediately packed in solid carbon dioxide in cardboard cartons, which were then sealed with tape. The material from the greenhouse was placed in thermos jugs and taken directly to a cold room maintained at  $38^\circ\text{F}$ . The sap was extracted from the samples by grinding the leaves in a meat chopper. Suspended matter was removed from the expressed sap by centrifugalization, and the moisture content was determined by means of a refractometer.

Such physicochemical properties as depression of the freezing point, osmotic concentration, and percentage of bound water were determined by the standard method (8). In this method,  $\Delta$  equals the freezing-point depression of the sap as determined by the Beckmann thermometer and  $\Delta a$  equals the freezing-point depression of a volume of sap sufficient to contain 20 g of water, in which 6.845 g of sucrose (a molar concentration) has been dissolved. The difference between these two values is expressed by the formula

$$\Delta a - \Delta = \Delta s.$$

If, then, the depression of the freezing point of a molar solution of sucrose alone is subtracted from  $\Delta s$ , this value equals  $\Delta x$ . From these values the bound water content, or hydrophilic colloidal content, of the sap is calculated by means of the following formula:

$$\Delta x.89.2 \left( \frac{\text{Percentage of free water in a molar solution of sucrose}}{\Delta s} \right) = \text{Percentage of bound water.}$$

#### EFFECTS OF HALOGEN SALTS ON THE TOBACCO PLANT

Tobacco responds readily to applications of salts containing chlorine, bromine, or fluorine, but the effects of the various compounds differ somewhat. When a mixed fertilizer containing 4 or 5 per cent of

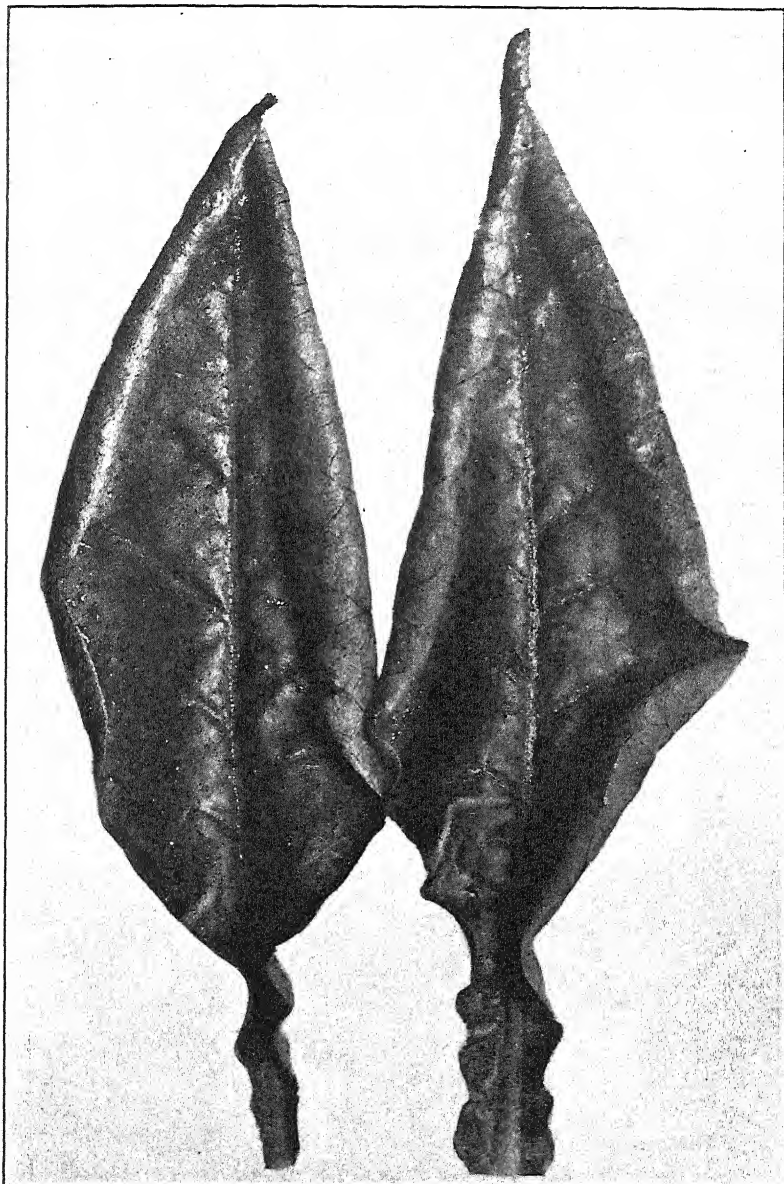


FIGURE 1.—Tobacco leaves showing injury from excessive application of chlorides

potash as potassium chloride was applied to tobacco plants in the field, these plants became stunted and their leaves were characteristically thickened and curled along the margin (fig. 1), thus confirming

the observations of Garner and his associates (4). In the greenhouse, in a soil which was deficient in nitrogen, potash, and phosphoric acid but to which chlorides were applied in excess, tobacco plants

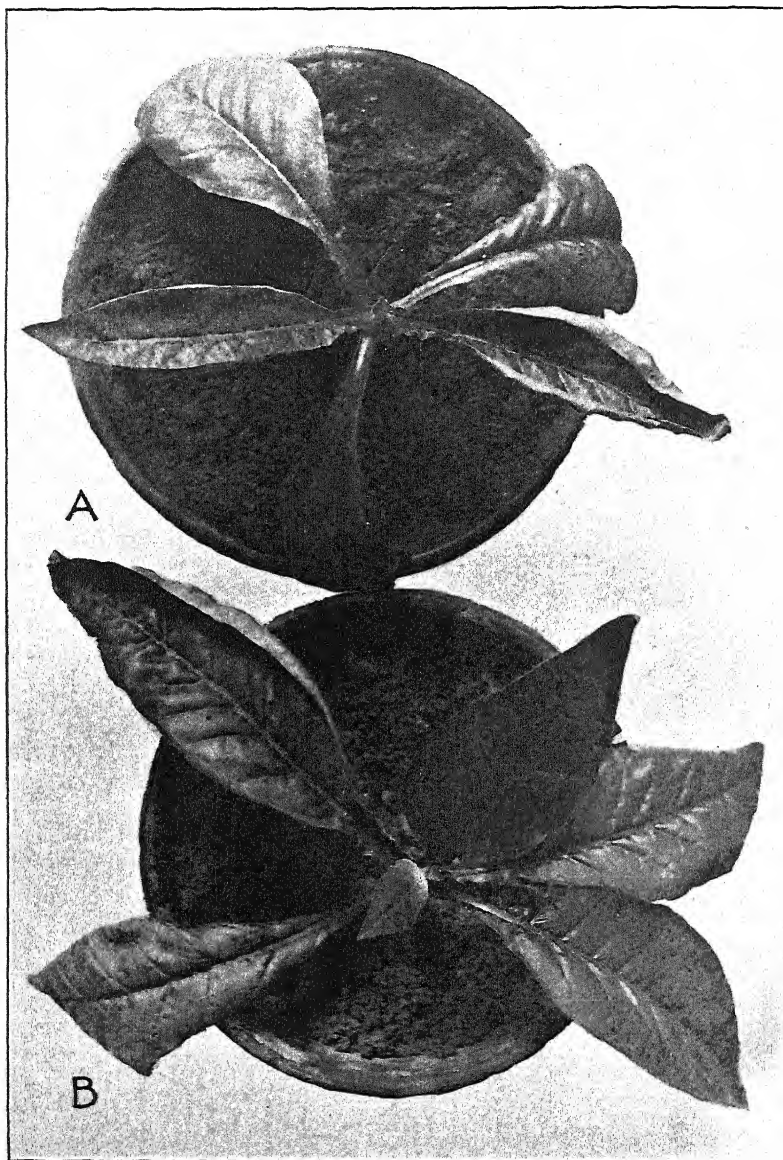


FIGURE 2.—Tobacco grown in the presence of bromides: A, Plant given applications of ammonium bromide; B, plant given applications of potassium bromide

grew less rapidly than did the controls. Instead of being thin and pliable, as normally, the leaves were thick and stiff. There was no curling upward of the leaf margins after applications of the chlorides of magnesium, potassium, or sodium; curling occurred, however, after



applications of ammonium chloride. From this effect it would appear that the cumulative action of an excess of chlorine alone does not cause curling. The fact that plants receiving applications of ammonium chloride made a more vigorous growth and had larger leaves than did those receiving the other chlorides indicates that the marginal curling of leaves may be conditioned by the availability of nitrogen and is therefore correlated with rate of growth.

Plants receiving applications of bromine as bromides of ammonium, sodium, or potassium developed leaves that were thick, shiny, and

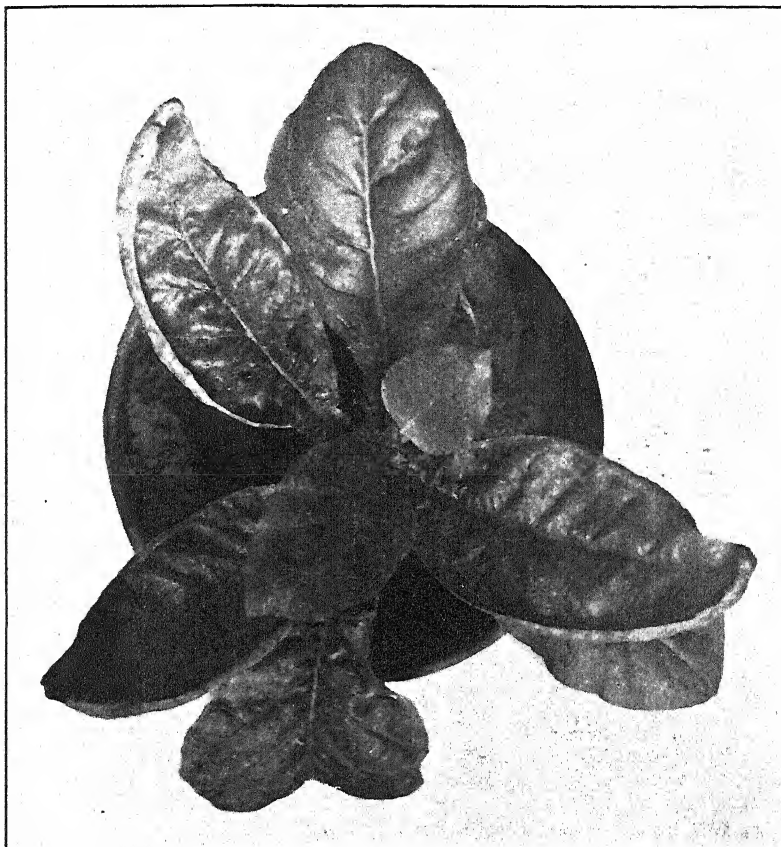


FIGURE 3.—Tobacco plant to which ammonium fluoride was applied

brittle. Curling of the leaf margin resulted from the use of ammonium bromide or potassium bromide (fig. 2), but not from the use of sodium bromide.

When permitted to accumulate in excessive amounts, fluorine produces a more pronounced inhibition of growth than does either chlorine or bromine; and it seems, in addition, to have a toxic effect. Marked thickening occurred after applications of fluorides of ammonium, calcium, potassium, or sodium; but there was no rolling upward of the leaf margins except after applications of ammonium fluoride. (Fig. 3.)

These results indicate that excessive amounts of chlorine, bromine, or fluorine interfere with the normal growth and expansion of tobacco leaves and with the elongation of the stems. From the chemical similarity of these elements, it may be assumed that their salts would have similar effects on cellular activities. Evidence of similarity in physiological effects on tobacco is shown by anatomical studies.

#### EFFECT ON ANATOMY OF LEAVES

Normal tobacco leaves embedded in paraffin were cut transversely in sections of sufficient thickness to include entire cells. Leaves from

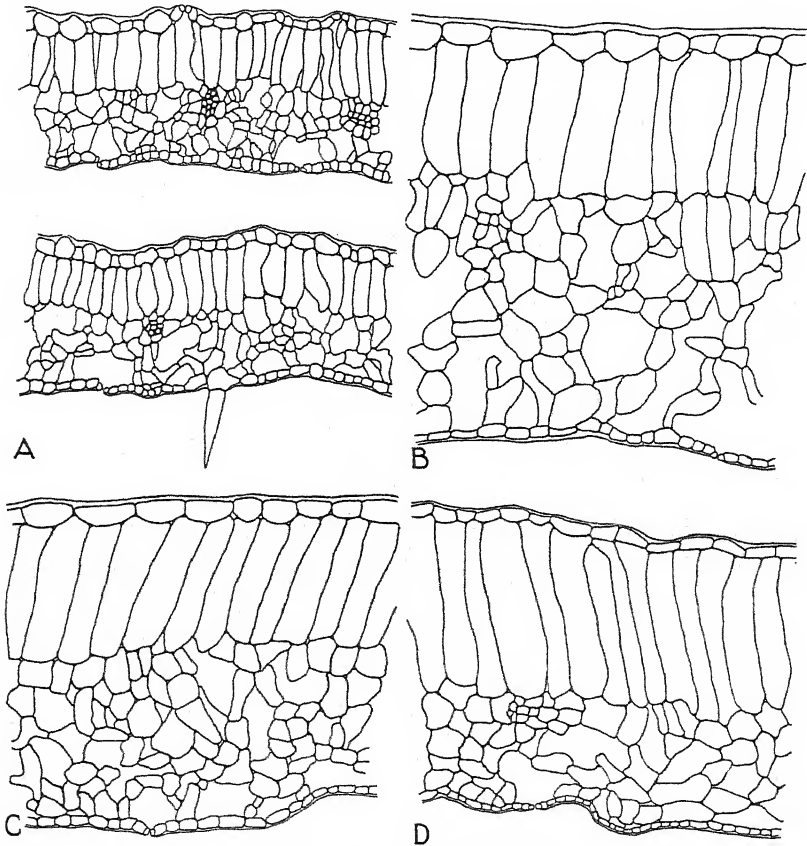


FIGURE 4.—Cross section of tobacco leaves showing thickening produced by halogen salts: A, Normal leaf; B, leaf injured by chlorine; C, leaf injured by bromine; D, leaf injured by fluorine

plants that had been given excessive applications of halogen salts were similarly embedded and sectioned. Microscopic examination of these sections showed that the salts had caused an increase in the thickness of the leaves and that this increase was due not to an increase in the number of cells but to the enlargement of cells of all tissues except, perhaps, those of the vascular bundles.

Figure 4 shows cross sections of leaves drawn to the same scale with the aid of a microprojector and indicates that leaves from plants

to which chlorides had been applied were approximately two and one-half times as thick as normal leaves, and that those to which bromine and fluorine had been applied were approximately twice as thick as normal leaves.

Garner and his associates (4) have shown that the increased thickness and brittleness of leaves of plants receiving an excess of chlorides is primarily due not to toxicity but to an interference with the carbohydrate metabolism in which the water relations are involved. As already stated, these investigators noted that an excess of chlorine causes the leaves to become gorged with starch. In earlier studies (2) on the curing of tobacco they reported that the partial wilting of the green leaf has a decided effect in stimulating amylolytic processes involved in the conversion of starch to reducing sugars. In the same year (1914), a similar starch-water relationship in plant tissues was noted by Lundegårdh (7), who found, furthermore, that an increase in water content causes starch to re-form. More recently Schroeder and Horn (9) and Bruns (1), in investigations on several species of plants, have confirmed the observation that starch is digested in cells which are not turgid and that it is re-formed when the cells have again absorbed sufficient water to become turgid.

The presence of chlorides in excessive amounts should therefore tend to maintain a high state of turgidity within the cells of tobacco leaves. It seems probable that bromides and fluorides would have a similar effect.

#### EFFECT ON PHYSICOCHEMICAL PROPERTIES OF SAP

In order to ascertain whether halogens modify the osmotic concentration of the sap and whether these changes are correlated with increased size of the leaf cells, determinations were made of the freezing-point depression, osmotic concentration, and bound-water percentage of extracted sap. The results of a series of such determinations are given in Table 1.

TABLE 1.—*Effects of various halogen salts on physicochemical properties of the sap of leaves of tobacco plants grown in the greenhouse in 1931*

Date of analysis	Solution applied	Freezing-point depression		Osmotic concentration	Bound water <sup>c</sup>
		$\Delta^a$	$\Delta^b$		
		<sup>o</sup> C.	<sup>o</sup> C.	Atmospheres	Per cent
July 9	Control (tap water).....	0.682	2.209	8.22	0
	KBr.....	.875	2.360	10.54	0
	NH <sub>4</sub> Br.....	.814	2.291	9.80	0
	KCl.....	1.092	2.845	13.14	0
	NaCl.....	.981	2.469	11.89	0
	NH <sub>4</sub> Cl.....	.941	3.046	11.33	0
	Control (tap water).....	.721	2.873	8.68	1.28
	KBr.....	1.025	3.201	13.06	0
July 21 and 22	NaBr.....	1.027	2.784	12.36	0
	NH <sub>4</sub> Br.....	.915	3.041	11.02	.21
	KCl.....	1.106	3.312	13.31	3.44
	NaCl.....	1.021	3.222	12.29	3.24
	NH <sub>4</sub> Cl.....	.903	3.064	10.87	1.65
	NaF.....	.788	2.992	9.49	3.36

<sup>a</sup> Freezing-point depression as determined by the Beckmann thermometer.

<sup>b</sup> Freezing-point depression of a volume of sap sufficient to contain 20 g of water in which 6.845 g of sucrose has been dissolved.

<sup>c</sup> Computed from freezing-point-depression values. (See p. 890.)

TABLE 1.—*Effects of various halogen salts on physicochemical properties of the sap of leaves of tobacco plants grown in the greenhouse in 1931*—Continued

Date of analysis	Solution applied	Freezing-point depression		Osmotic concentration	Bound water
		$\Delta$	$\Delta a$		
		$^{\circ} C.$	$^{\circ} C.$	<i>Atmospheres</i>	<i>Percent</i>
Aug. 3	(Control (tap water) .....	0.708	2.895	8.53	2.81
	KBr.....	1.082	3.250	13.02	2.06
	NaCl.....	1.009	3.181	12.15	2.22
	NaF.....	.874	3.056	10.52	2.62
Aug. 14	(Control (tap water) .....	.817	3.043	9.84	4.25
	NH <sub>4</sub> Br.....	.876	3.079	10.55	3.36
	KCl.....	1.126	3.288	13.55	1.73
	NaF.....	.959	3.192	11.55	4.51
Average	(Control (tap water) .....	.732	2.755	8.82	2.05
	KBr.....	1.014	2.937	12.21	.69
	NaBr.....	1.027	2.784	12.36	0
	NH <sub>4</sub> Br.....	.868	2.804	10.46	1.19
	KCl.....	1.108	3.148	13.33	1.72
	NaCl.....	1.004	2.957	12.11	1.82
	NH <sub>4</sub> Cl.....	.922	3.055	11.10	.83
	NaF.....	.874	3.080	10.52	3.50

These data show that sap from the leaves of plants receiving excessive amounts of salts had larger values for  $\Delta$  and  $\Delta a$  than that from the leaves of plants receiving tap water only, or that the cell sap of plants to which salts had been applied had the greater osmotic concentration.

Garner and his associates (3) have shown that there is a progressive increase in hydrogen-ion concentration in the sap of tobacco leaves as the leaves approach maturity. Since active acidity is correlated with changes in osmotic concentration there should also be a gradual increase in osmotic concentration in plants that are approaching maturity and that have received applications of salts. The data in Table 1 indicate a tendency toward increased osmotic concentration with increasing age.

The ability of the leaf to bind water is influenced by an excess of salts, and also by the age of the leaf as indicated by the absence of bound water in all samples on July 9 (Table 1) and by its occurrence in nearly all later samples. Further evidence of the influence of age as a factor in increasing the binding of water was strikingly exhibited by the difficulty with which sap could be extracted from leaves gathered from the field early in October, 1931. The present data, with the exception of the results obtained when the plants were given sodium fluoride, show that the bound-water content of normal leaves is higher than that of the leaves of plants to which chlorides or bromides has been applied. The physicochemical effects produced by sodium fluoride indicate that the toxicity of this salt may possibly have interfered with the absorption of water, thus causing more than the normal quantity of free water to be transformed to bound water and producing a condition similar to that which occurs during drought. While studying drought resistance in cereals, Harris and his co-workers, in an investigation cited by Gortner (5, p. 235-236), noted that as long as there is an abundance of moisture there is little tendency for the development of hydrophilic colloids and the elaboration

of bound water but that with the onset of conditions of stress, as of drought, water is transformed from the free to the bound state.

The moisture supply of tobacco plants growing in the field fluctuates over a wide range during the season. In the present experiments the moisture supply of plants grown in the greenhouse was maintained more nearly at the optimum. In physicochemical properties the sap of plants grown in the field may therefore differ from that of those grown under glass. Data bearing on this question were secured by comparing the results of the greenhouse experiments (Table 1) with results obtained from tobacco leaves collected in the field in each of the two localities previously mentioned, namely, area 1, north of Durham, N. C., and area 2, south of Durham (Table 2).

TABLE 2.—*Effects of chlorides on physicochemical properties of the sap of leaves of tobacco plants grown near Durham, N. C., 1931*

AREA 1 (NORTH OF DURHAM)

Date of analysis	Effect of indicated quantity of chlorides on—							
	Freezing-point depression				Osmotic concentration		Bound water	
	$\Delta a$		$\Delta a^b$					
	Normal Cl	Excess Cl	Normal Cl	Excess Cl	Normal Cl	Excess Cl	Normal Cl	Excess Cl
	<i>° C.</i>	<i>° C.</i>	<i>° C.</i>	<i>° C.</i>	<i>Atmospheres</i>	<i>Atmospheres</i>	<i>Per cent</i>	<i>Per cent</i>
June 11.....	0.968	0.982	3.237	3.262	11.66	11.82	3.89	4.30
June 23.....	.590	.591	2.800	2.765	7.11	7.12	2.74	1.31
July 10.....	.706	.676	2.250	2.175	8.50	8.14	0	0
July 24.....	.828	.803	3.214	3.041	9.97	9.67	11.18	6.02
Average.....	.773	.763	2.875	2.811	9.31	9.19	4.45	2.90

AREA 2 (SOUTH OF DURHAM)

June 18.....	0.618	0.567	2.842	2.718	7.45	6.83	3.29	0.37
June 24.....	.785	.848	3.096	3.087	9.45	10.21	6.52	3.86
July 11.....	.643	.643	2.130	2.024	7.75	7.75	0	0
July 25.....	.891	.666	3.160	2.847	10.73	8.02	7.15	3.84
Average.....	.734	.681	2.807	2.669	8.85	8.20	4.24	2.02

<sup>a</sup> Freezing-point depression as determined by the Beckmann thermometer.

<sup>b</sup> Freezing-point depression of a volume of sap sufficient to contain 20 g of water in which 6.845 g of sucrose has been dissolved.

<sup>c</sup> Computed from freezing-point-depression values. (See p. 890.)

In each of these two areas tobacco leaves were collected from two fields, in one of which chlorides had been applied in excess, whereas in the other only the normal 2 per cent or less of chlorides in the fertilizer had been applied. The chlorine-injured leaves selected for examination, all of which were taken from stunted plants, were curled and exceedingly brittle.

Table 2 shows that on corresponding dates the freezing-point depression, the osmotic concentration, and the bound-water percentage were, in general, greater in the normal leaves than in those injured by an excess of chlorides.

Moreover, the osmotic-concentration values of field-grown plants to which excessive applications of chlorides were made (Table 2) were not the same as those of plants grown under glass in the presence of an excess of sodium chloride or potassium chloride (Table 1). Such differences may be explained by the fact that there was less fluctuation in the water supply of plants grown under glass than in that of plants grown in the field. Since "normal" plants (Table 2) were liberally supplied with essential minerals and the controls (Table 1) were grown in a soil deficient in minerals, the properties of the sap of these normal and control plants can not be expected to accord.

It might be expected that the values for normal leaves in Table 2 would exceed those of leaves injured by chlorine. The apparent inconsistencies may be partly due to lack of correspondence in age of the normal and the chlorine-injured plants. Both normal and chlorine-injured plants from which leaves were taken for the analyses recorded in Table 2 were transplanted at approximately the same date. In both tables is shown a tendency toward increase in osmotic concentration with increase in age of plants. It is well known among growers that the leaves of plants given an excess of chlorine mature later, by two weeks or more, than those of normal plants. Despite the apparent equality in age, the chlorine-injured plants remained considerably smaller and hence were physiologically younger than normal plants set at the same date.

Moreover, as previously stated (4), it has been found that excess of chlorine results in a low organic-acid content. The differences between the osmotic-concentration values of the sap of normal leaves and those of the sap of leaves of chlorine-injured plants may, therefore, be occasioned by the relatively high acid content of normal leaves and the low acid content of the chlorine-injured ones.

So far as bound water is concerned, all data are in agreement in showing that there is relatively little tendency for the transforming of water from a free to a bound condition when chlorine is present in excessive amounts.

#### SUMMARY

Previous investigations have shown that moderate applications of chlorine stimulate the growth and increase the yield of tobacco. An excess of chlorine, however, interferes with normal carbohydrate metabolism and causes the leaves to become thick and brittle and to curl upward at the margins. Such leaves have a high starch content, a high water content, and a low organic-acid content.

The present anatomical studies of leaves from normal tobacco plants as compared with leaves from plants to which certain chlorides, bromides, or fluorides were applied show that these elements cause the leaves to become thickened by enlargement of the leaf cells. When the concentration of the halides is maintained at approximately the limit of tolerance, chlorine causes greater enlargement than does bromine or fluorine.

Experiments with plants grown under glass in the presence of an excess of a single salt show that chlorine, bromine, or fluorine increases the osmotic concentration of expressed sap, as measured by depression of the freezing point. Increased osmotic concentration in tobacco is therefore correlated with enlargement of the leaf cells. The osmotic-concentration values tend to increase with the age of the plant.

The leaves of tobacco plants to which excessive amounts of chlorides or bromides have been applied have less bound water than the leaves of plants grown in a medium deficient in mineral nutrients.

Tobacco responds to sodium fluoride by transforming free water to bound water in such quantities as to indicate that this compound induces a state of physiological drought.

The sap of tobacco plants grown in the field in the presence of excessive amounts of chlorides had a lower osmotic concentration and less bound water than the sap of field-grown plants to which a liberal application of a complete fertilizer had been given. The lower values of the sap of chlorine-injured leaves may be partly explained by physiological immaturity and low organic-acid content.

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# INFECTIOUS VARIEGATION IN THE APPLE<sup>1</sup>

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## INTRODUCTION

The variegation of apple leaves discussed in this paper appeared as an unexpected complication in an experiment on double working, raising new questions which had to be answered before the study as originally planned could proceed with any prospect of definite results. Since this secondary study has reached a point where separation of horticultural and pathological aspects seems possible, and since it deals with a matter which has received rather scant attention, its salient points are placed on record, together with such historical data as are available.

## HISTORY OF THE DISEASE

Under various names, variegation of apple leaves has been mentioned several times in the literature. Stewart (12),<sup>3</sup> in 1910, described sporadic cases found in New York State since 1896. In 1915 Clinton (5) described a manifestation in Connecticut which may have been identical. In the following year Morse (9) reported, as a leaf trouble "new to Maine" the occurrence of chlorotic areas in leaves of Baldwin, Northern Spy, and Harvey. The appearance of the leaves shown in his photographs is in every way similar to that of the diseased leaves discussed in this paper. Blodgett and others<sup>4</sup> have reported as "apple mosaic" additional cases in New York, and have demonstrated that the disease is transmissible by budding and grafting. Blodgett states<sup>5</sup> that specimen leaves from material used in the study here reported "certainly look identical" with the trouble he reported, that Whetzel has collected similar specimens in Michigan, and that others who have seen his specimens have recalled seeing similar cases in other States.

In northern Germany Braun (2) reports as "Buntblätterigkeit" or "Panaschierung" the occurrence in 1928 of "white spots in otherwise sound green leaves" in scattered trees of the apple varieties Fettaffel and Rosenapfel.

Though a rather extensive search of the classic papers on infectious variegation yields no mention of this disease in the apple, it is not to be inferred that it is particularly new. Inasmuch as the early European reports have been overlooked or discredited, and inasmuch as they should constitute a rather important chapter in the history of infectious variegation, a detailed statement is presented here.

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<sup>2</sup> The writers acknowledge their indebtedness to W. C. Dutton, of the Michigan Agricultural Experiment Station, for the photograph used and to Dr. Ray Nelson, of the Michigan station, and Dr. F. M. Blodgett, of Cornell University, for references to American phytopathological literature.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 908.

<sup>4</sup> ORTON, C. R., and WOOD, J. I. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 33 : 82. 1924.

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In 1867 Bossin (1), without giving citation, stated that Vibert had described a variety of apple with variegated leaves. This, according to Bossin's recollection, occurred about 1827. Bossin made no mention of the variegation being infectious, though the nature of his topic gave him occasion to note a fact of this nature.

In 1830 Sageret (11) stated that he had read in the Philosophical Transactions an account (presumably Cane's (3) published in 1720, describing an observation made in 1692) of transmission of variegation from scion to stock in jasmine. In 1825 Noisette (10), whose nursery was near Paris, had reported observing, on several occasions, transmission of variegation from scion to stock in several kinds of woody plants, which he did not specify. Apparently Sageret had not seen Noisette's statement, for he mentioned the account of the jasmine without, as he said later (reported by Duchartre (6)), giving it full credence, because he knew of no parallel case. Vibert, upon seeing Sageret's statement concerning the jasmine, reported to him that he had seen transmission of variegation in the apple. Some years later Vibert (13) gave an account of this occurrence, as follows:

Some time ago, and during several years, I have seen, at the establishment of a brother of Noisette, the nurseryman, at La-Queue-En-Brie, apple trees which, having been shield-budded with varieties with variegated leaves, and their grafts failing from any cause whatever, none the less produced branches with variegated leaves. Astonished at this strange anomaly, I repeated at home the same operation, on Paradise, using as scion the pommier à feuilles d'Aucuba. In the following spring I completely destroyed all my scions, and all the stocks produced shoots with leaves more or less variegated, even those whose grafts had perished after being inserted.

Vibert recorded at the same time a similar subsequent experience with the rose on dog-rose stock.

In 1839, according to Leroy (7, v. 3, p. 301-302), the nurseryman Noisette gave the initial description of the "pommier à feuilles d'Aucuba," so called because of its variegated leaves, and Leroy secured specimen trees from the vicinity of Paris at that time. Noisette's mention in 1825 of transmission, his initial description of a variegated variety, and his brother's experience seem to warrant the belief that the apple was one of the plants on which he based his 1825 report. If it was not, Vibert had demonstrated transmission in the apple by 1835, when Sageret reported Vibert's statement, as mentioned by Duchartre (6).

Sageret, having been somewhat sceptical of the jasmine case, encountered similar scepticism of his own report concerning the apple. Morren (8) stated some years later:

Pyrame de Candolle, Poiteau, and others of the time attached true importance to it, but without being convinced of its authenticity. Sageret's observation was never cited except with hesitation. The new facts [i.e., those available in 1870] established the truth of the phenomenon in an incontestable manner.

Apparently Sageret's report came too early. Had it been made in 1870 its historical importance in antedating nearly all of the better known cases would undoubtedly have gained wider recognition.

Other reports indicating infectious variegation in the apple are rare and vague. A specimen submitted by Simirenko, as reported by Carrière and André (4) in 1889 suggests a manifestation of infectious variegation, but is not evidence. Consideration should, however, be given the fact that trees raised in well-conducted nurseries have little opportunity of manifesting variegation in the seedling roots where the

potentiality for manifestation is greater. Leroy cultivated the pomier à feuilles d'Aucuba in his nursery at Angers, and stated in 1873 that it was more notable for its variegation than valuable for its fruits and that nurseries were no longer interested in it, but he made no mention of infectiousness of the variegation. Leroy's training and interests seem to warrant the inference that had he noted the occurrence he would have recorded it. If the variegation in Leroy's trees was not infectious, Noisette's trees of the same variety manifested the not impossible but highly improbable coincidence of infectious variegation with a noninfectious variegation. The probability is, therefore, very strong that Leroy cultivated infection-carrying trees without noting the infectious nature of the variegation. Others may have done the same thing.

#### METHODS

In the spring of 1928 the Department of Horticulture of the Michigan Agricultural Experiment Station began an experiment designed to determine whether the practice, rather common among nurserymen, of working certain weak-growing varieties of pear and apple on vigorous intermediate varieties has any effect beyond making a good nursery tree. For stocks, 2-year-old apple seedlings, raised at East Lansing in 1926 and lined out for one year, were used. These were grafted close to the ground (but high enough to preclude scion rooting) with scions of the Red Canada (Steele Red <sup>6</sup>) apple and of other varieties designed to serve as intermediates to Steele. The Steele scions were secured from an orchard at Ovid, Mich., where they were grown as top grafts on Tolman Sweet. The further history of this strain is not known beyond the fact that the scions were secured by the late T. A. Farrand from an orchard which he described as the best Steele orchard in Michigan. No other Steele grafts or buds were used in the nursery until 1931.

During the growing season of either 1928 or 1929, small spots, some yellow and some cream white, of irregular outline (fig. 1), were noted on some leaves of the Steele grafts, but no significance was attached to them, and the work of building double-worked trees went ahead in 1929 and 1930, according to the original plan. When the leaves opened in 1930, a seedling which had been unsuccessfully grafted with Steele in 1928 showed yellow spotting in a very pronounced degree. Further examination revealed several other seedlings similarly affected; in all cases, however, they were seedlings which had been grafted to Steele, unsuccessfully. Variegation was found on a considerable number of living Steele scions, and in a few—not all—cases where seedling sprouts had not been completely eliminated variegation was found on their leaves. On a few trees which had been root-grafted in 1927 and top-grafted to Steele, with scions of the same origin, variegation was found in Steele leaves, in leaves of the Nixonite intermediate, but not in leaves of Virginia Crab used as an intermediate. Careful scrutiny of every tree in the nursery revealed no variegation in any tree except some of those which had been grafted, successfully or unsuccessfully, to Steele.

The manifestly infectious nature of this variegation raised two questions: (1) Whether the disease is endemic in the Steele variety and (2) whether other varieties are resistant or immune.

<sup>6</sup> In Michigan two distinct strains of Red Canada have been recognized, and one has been designated Steele, the name used in this paper. The material used was supposedly of the Steele strain.

The belief that the disease might be endemic was strengthened by vague reports that this variegation is more or less characteristic of Steele in the nursery. Though an inspection of all trees of this variety in two large nurseries in June, 1930, revealed no trace of variegation, the question was not regarded as definitely settled inasmuch as variegation had been found on seedling sprouts of trees whose Steele leaves showed no trace of it, and this seedling index was unavailable in most of the nursery trees. In addition, the usual slow growth of Steele trees and the common lack of vigor in variegated plants seemed to lend some support to this view. Accordingly, in the spring of

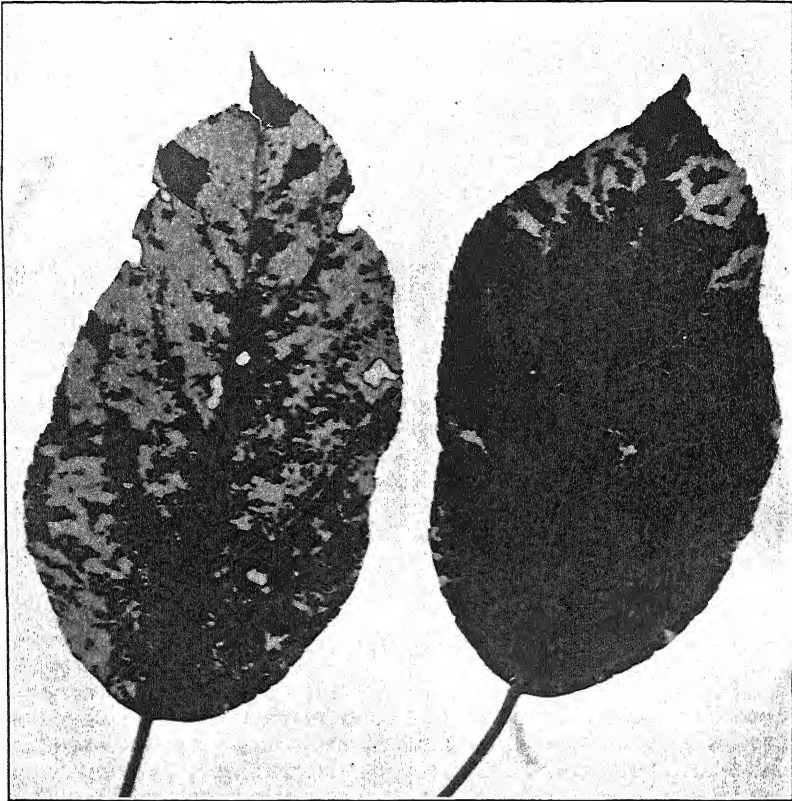


FIGURE 1.—Apple leaves showing infectious variegations

1931, scions from two other orchards, as well as those tracing to the original diseased strain, were used. Grafts of several other varieties were set on seedlings, high enough to permit retention of seedling leaves as indicators of transmission. These were budded in the ensuing August, record being kept of the source of buds in each case. With the unfolding of the leaves in 1932, the effects of these various manipulations could be appraised.

In 1930 variegation was recorded by the senior author; in 1931 and 1932 by the junior author. Since differentiation between very small spots due to variegation and others due to insects is a rather nice matter, the junior author made his diagnosis in ignorance of the

history of the trees, which were marked only by jumbled numbers. This removal of personal bias makes some of the findings particularly striking. Variegation was arbitrarily recorded as "trace", "moderate", and "heavy." Though no rigid standard was maintained, classification was made on the basis of conspicuousness, which, analyzed, signifies the development of large variegated areas rather than number of leaves affected.

### RESULTS OF EXPERIMENTS

Three-year records (1930-1932) are available for 109 trees of Steele worked directly on seedlings. Of the 40 which showed variegation in the first year, 33 have shown it in the other two. Of the 69 which showed no variegation in 1930, 47 showed it in 1931; of the remainder, 11 showed it in 1932. Only 11 have shown no variegation in any of the three years. It is possible that some of these are free from variegation, since four buds from one of them set in Fameuse grafts in 1931 failed to produce variegation, a sequence not equaled in any budding from trees obviously infected. The increase in percentage of trees affected from 1930 to 1931 is not interpreted as evidence of the spread of the disease from tree to tree, for trees not grafted to Steele, although intermingled freely with those that were grafted to Steele, in no case developed the disease.

In these first trees propagated before variegation was recognized, efforts were made to prevent development of sprouts from the seedling stocks. Consequently data on transmission to the stocks must be drawn from observations on generally very reduced sprouts on 40, 35, and 26 trees in the respective years. The small number of growing points on these sprouts obviously reduced the chances of observation. In those cases in which observation was possible, however, variegation was found in 15 per cent in 1930, 34 per cent in 1931, and 19 per cent in 1932.

More reliable figures on transmission to seedlings are available from 93 grafts of the same strain set higher on seedlings in May, 1931. In June, 1932, 63 per cent of these scions and 46 per cent of their stocks showed variegation. No explanation is offered for the smaller percentage of variegation on the older trees in 1932 beyond the possible effect of transplanting on a portion of them. Those that were dug in the fall of 1931 and reset in the spring of 1932 showed much less variegation in the following summer.

Steele scions from another orchard were set in the spring of 1931. Of these, 109 were set on seedlings and 60 top-worked into various varieties, and in the following August 58 buds from these scions were set. In all, then, 257 opportunities were afforded this lot to show variegation; actually, 1 case was found. Accounting for this single case is not attempted; a mixture of scions is possible, since on one or two occasions the operator carried two lots at the same time. Nine scions from a third orchard failed to develop any visible variegation or to transmit it to young seedlings, and 112 of them top-worked into a mature tree failed to develop any symptom of variegation. Apparently, then, infectious variegation is not universal in this variety. On the other hand, buds taken from a mature Steele tree in the college orchard in 1931 developed variegation in stocks on which they were worked, and in shoots developing them, though examination of this tree in 1930, 1931, and 1932 revealed no variegation.

The best comparable data available on varietal susceptibility is that afforded by the grafts and buds set in 1931, as recorded in June, 1932. These are presented in Table 1. These percentages would probably be greater if the experiment were continued another year, but it appears at present that Tolman Sweet and Northern Spy are much less inclined to show variegation than the other varieties. Not only is the percentage of visibly affected trees smaller, but the amount of variegation as measured by percentage of leaves and area per leaf involved is distinctly lower.

TABLE 1.—*Appearance in 1932 of infections variegation in apple trees of several varieties top-worked with infected Steele scions in 1931*

Intermediate variety	Trees observed	Variegation shown in—		
		Steele scion	Inter- mediate	Seedling stock
	Number	Per cent	Per cent	Per cent
None (worked direct).....	93	63		46
Tolman Sweet.....	44	57	9	9
Wolf River.....	19	95	47	44
Northwestern Greening.....	14	100	79	21
Fameuse.....	69	64	42	22
Delicious.....	17	77	53	(a)
Yellow Bellflower.....	10	50	40	(b)
Oldenburg (Duchess).....	9	100	38	38
Northern Spy.....	16	56	0	13
Virginia Crab.....	26	71	46	(c)

<sup>a</sup> Root-grafted.

<sup>b</sup> No observations possible.

The danger of attaching significance to one year's results is illustrated by the Virginia Crab. Eight nursery trees of this variety were top-worked in 1928 with Steele scions which subsequently proved to be variegated; others were top-worked with similar scions in 1930 and 1931. Variegation appeared in the Steele scions rather consistently in 1930 and 1931, but none was found in Virginia Crab, and until the leaves unfolded in 1932 this variety was regarded as immune. In these earlier propagations comprising 35 trees the sole case of variegation in Virginia Crab occurred in 1932. The figures presented in Table 1 represent a later propagation and present a totally different condition.

The lower percentage of seedling stocks showing variegation, as contrasted with the grafts set on them, has been attributed to the smaller number of leaves available for observation, rather than to resistance. This view is taken because of their more pronounced manifestation of variegation when it does occur. Without exception, the proportion of cases of pronounced variegation to all cases found was higher in seedling stocks than in the scions (Table 2). The small number of observations vitiates the results with Oldenburg, Northwestern Greening, and Northern Spy, but the consistency of the direction of the differences compensates in some measure for the paucity of material. Apart from this consideration, any attempt to differentiate between conditions which might be distinguished as susceptibility, manifestation of infection, tolerance, or resistance would be unwarranted on the basis of the evidence here available. Since varieties appear to differ, at least in their manifestation, seedlings probably do likewise. Consequently triple-worked trees, or

clonal rootstocks, would be necessary for further progress in this direction.

TABLE 2.—*Percentages of variegation cases classified as pronounced.*

Name of intermediate	Steele	Intermediate	Root
Direct.....	25	-----	36
Fameuse.....	18	18	58
Tollman Sweet.....	25	0	50
Wolf River.....	22	11	88
Oldenburg.....	22	0	33
Northwestern Greening.....	6	8	33
Northern Spy.....	0	(a)	50
Combined.....	20	13	46

<sup>a</sup> No variegation recorded.

Transmission from stock to scion has occurred. Scions uninfected when set in 1931 into a mature tree, which in 1930 had shown a slight evidence of variegation, were rather heavily infected in 1932, though traces on the stock were still very inconspicuous. Variegated scions set in mature trees produce variegation which spreads throughout the tree, even to the tips of ungrafted limbs.

No indication of rapid spread of this disease from tree to tree has been observed. With the one exception already noted, no case has been found in the nursery which could not be traced to a graft, successful or unsuccessful, with infected scions. Infected and uninfected trees have been intermingled thoroughly, and no effort at insect control has been made. Spread through meeting and natural grafting of roots seems entirely possible, since sprouts originating on roots below ground have shown variegation, but no such case has been observed.

No opinion can be advanced here as to the effect of this disease. Two mature variegated trees are dying back at the tips of the branches; two others are distinctly subnormal, but since these trees have other troubles their weak condition can not be definitely attributed to variegation. In the nursery, variegated trees have shown no noticeable diminution of vigor.

Other cases showing identical symptoms have been observed. Two mature trees, one of the variety Marks and the other unidentified, in the college orchard have given clear, if inconspicuous, indication of the disease; a tree of Star has become infected by infected grafts, and a clon of root sprouts taken in 1926 from a tree since removed from the college orchard is thoroughly infected. Single cases occur in other parts of Michigan, involving mature Baldwin, Wolf River, and Fameuse trees; in one case variegated leaves were sent in as specimens of spray burn. Transmission from variegated Baldwin scions through a mature Wolf River tree to other Baldwin scions has occurred at the Graham Horticultural Experiment Station, Grand Rapids, Mich.

Attention is called to the apparent suitability of the apple as material for fundamental investigation of infectious variegation. The large number of clonal varieties with clear and well-recognized differences in vigor and responsiveness to environmental conditions, together with the general ease of handling the apple in the field and

in the laboratory, provide an unusually good foundation for pathological studies.

### SUMMARY

Attention is called to the fact that one of the first cases of infectious variegation recorded was in the apple.

Varieties of apple differ, if not in susceptibility, at least in manifestation of infectious variegation.

None of the varieties tested is regarded as wholly immune.

Seedling stocks showed higher proportions of pronounced manifestations of this disease than did the varieties worked on them.

Buds taken from a tree showing no variegation produced it in seedling stocks.

Isolated apparent cases of variegation on several varieties are mentioned.

The effect of the disease on the tree has not yet been determined.

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# EXPERIMENTS ON THE CONTROL OF SEED-BORNE DISEASES BY X RAYS<sup>1</sup>

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## INTRODUCTION

Seed-borne diseases are those whose causal organisms are carried either within, or upon the surface of, the dormant seeds. The plants from infected seeds may develop serious seedling infections or, in case the disease is systemic, disorders in the mature plants. There are a number of crop seeds which carry the causal organisms of economically important diseases.

The losses resulting from seed-borne diseases vary, depending on the areas under consideration and the care used in seed selection. In some cases they have been estimated at as much as 10 per cent of the crop (2).<sup>2</sup> Among the organisms that infect corn, four were selected for the purpose of this study: *Diplodia zeae* (Schw.) Lév., *Gibberella saubinetii* (Mont.) sacc., *Fusarium moniliforme* Sheld., and *Cephalosporium acremonium* Cda. Of these *D. zeae* and *G. saubinetii* are probably the most serious, especially from the standpoint of corn-seedling injury. Other organisms studied were loose smut, *Ustilago tritici* (Pers.) Rostr., and bunt, *Tilletia levis* Kühn, of wheat; loose smut, *U. nuda* (Jens.) Kell. and Sw., of barley; and loose smut *U. avenae* (Pers.) Jens., of oats.

The recommended control of these organisms includes the application of germicides to the seed surface, and temperature treatments. Carefully conducted tests have indicated that organic mercury compounds are valuable in the control of certain organisms attacking corn. Copper carbonate and formaldehyde are widely used in the control of certain cereal diseases, and hot-water treatments have been used successfully in controlling loose smut of wheat.

The problem of controlling seed-borne organisms with X rays involves a study of the host reaction as well as that of the parasite. The X-ray treatment should not injure the host. Additional practical considerations are the efficiency of this treatment as compared with others and the cost. The use of X rays would permit treatment at any time before planting and would not unfit the seed for food purposes as do many chemicals.

## REVIEW OF LITERATURE

The results obtained by using X rays as the sterilizing agent have varied. Much work has been done on the treatment of bacteria, especially those parasitic on man. Only those references dealing with the irradiation of plants will be cited.

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 914.

Trumbull and Hotson (12) tried X rays as a means of controlling *Fomes pinicola* (Fr.) Cke. on logs of fir and western hemlock, but without success. Levin and Levine (5) inoculated a series of castor beans, *Ricinus communis*, with *Bacterium tumefaciens* EFS. and Town. and immediately afterwards subjected the inoculation site to X rays. Six treatments given at intervals of two days prevented the development of tumors, apparently with no injury to the host plants. Rivera (9) described the successful control of *Bact. tumefaciens* tumors on castor-beans and geranium plants. In a later paper (11) he concluded that the preventive action was a function of the effect of radiation on the host tissue rather than on the bacterial cells. Nadson and Philippov (7) found the vegetative tissue of *Mucor* more resistant to X rays than were the sporangia. Small doses appeared to stimulate the development of *M. genevensis*.

Rivera (10) irradiated cereal seeds heavily infected by smut fungi. The treated cereals produced 36.1 per cent infected spikelets, and the untreated produced 36 per cent. Pichler and Wober (8) obtained favorable control results with spores of *Tilletia tritici* (Bjerk.) Wint. The killing action was augmented by increasing the acidity of the medium in which the spores were irradiated, particularly in the presence of oxygen or oxygen-yielding substances. Irradiated seed appeared to be injured slightly by the treatments, but potato tubers treated for the control of *Chrysophlyctis endobiotica* Schilb. were not injured. Preliminary observations by Mulvania (6) showed that X-ray treatments on the virus of tobacco mosaic had no apparent effect. Johnson (4) found that *Sclerotium bataticola* Taub., *Collybia dryophila* Fr., and *Fusarium batatatis* Wr. were unaffected by X-ray treatments.

#### EXPERIMENTAL PROCEDURE

In the study reported in this paper two phases of the problem of controlling seed-borne diseases by X rays were considered—the reaction of pure cultures of the fungi, and the reaction of the host and associated parasite to X rays.

The pure cultures were obtained from the botany department of the University of Missouri, with the exception of *Diplodia zeae* which was isolated from a corn seed.

Streak transfers were made to thin layers of potato-dextrose agar in Petri dishes. The cultures were incubated from three to five days before irradiation. Immediately before irradiation the colonies in each Petri dish were cut into small squares (3 to 4 mm). During irradiation the Petri dish covers were removed and replaced by a washed photographic film. The four fungi, each in a separate Petri dish, were irradiated simultaneously. After treatment the squares were removed from the culture and transferred to a Petri dish containing potato-dextrose agar. The effects of irradiation were noted after incubating the squares eight days at 28° C.

An old model Kelly-Koett X-ray machine was used. Intensity measurements were made with a Victoreen iontoquantimeter. A description of the dose given in each treatment includes the peak voltage, milliamperage, distance of the target from the material being X rayed, the length of exposure, and the dose in International r units per minute.

Lots of corn seed infected with *Diplodia zeae* and *Gibberella savinetti* and nearly disease-free seed were obtained from Dr. J. R.

Holbert, Bloomington, Ill. The corn seeds for the isolation experiments were treated, germ side up, in a layer one seed deep. Those for the field experiments were exposed at random in a layer approximately three seeds deep.

The presence of internal parasites in the corn seeds was investigated by germinating the seeds on standard potato-dextrose agar at 28° C. The method used was similar to that of Chen (1).

The field plantings were in a fertile silt loam. The rows were 30 inches apart and the seeds 6 inches apart in the row.

The infected wheat, barley, and oats were secured from the Missouri experiment station.

#### TREATMENT OF PURE CULTURES OF FUNGI

Preliminary experiments with pure cultures of *Penicillium* sp., *Aspergillus* sp. and *Diplodia zeae* showed that exposure to X rays decreased the rate of growth of the fungi and that large doses caused death or inactivation. However, the doses required to kill the fungi in pure cultures seriously injured dormant corn seeds.

Cultures of *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and *Cephalosporium acremonium* were X rayed at different temperatures in order to establish their lethal doses and to determine the effect of temperature on the lethal doses. The cultures were exposed to the respective temperatures for 10 minutes preceding the treatment and during the period of treatment. Table 1 summarizes the effects of X rays, on the mycelium and spores of fungi at various temperatures.

TABLE 1.—Effect of X rays on mycelium and spores of fungi in culture at various temperatures as determined by the development of X-rayed transfers

[120 k. v. (peak), 4 ma., 16 cm target distance; 375 r units each minute]

Temperature (C°)	Fungus	Development after being X rayed for indicated period in minutes*									
		0	30	40	50	60	70	90	100	110	130
10	<i>Diplodia zeae</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Gibberella saubinetii</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Fusarium moniliforme</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Cephalosporium acremonium</i> .....	+	+	+	+	+	+	+	+	+	+
20	<i>Diplodia zeae</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Gibberella saubinetii</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Fusarium moniliforme</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Cephalosporium acremonium</i> .....	+	+	+	+	+	+	+	+	+	+
30	<i>Diplodia zeae</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Gibberella saubinetii</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Fusarium moniliforme</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Cephalosporium acremonium</i> .....	+	+	+	+	+	+	+	+	+	+
40	<i>Diplodia zeae</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Gibberella saubinetii</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Fusarium moniliforme</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Cephalosporium acremonium</i> .....	+	+	+	+	+	+	+	+	+	+
50	<i>Diplodia zeae</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Gibberella saubinetii</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Fusarium moniliforme</i> .....	+	+	+	+	+	+	+	+	+	+
	<i>Cephalosporium acremonium</i> .....	+	+	+	+	+	+	+	+	+	+

\* Plus sign indicates growth; minus sign no growth.

The resistance of *Fusarium moniliforme* and the susceptibility of *Diplodia zeae* indicate a wide range in the tolerance of different fungi. The order of susceptibility to X rays was *D. zeae*, *Gibberella saubinetii*, *Cephalosporium acremonium*, and *F. moniliforme*.

The susceptibility of the fungi to X rays was increased by exposure to high temperatures. Cultures of the fungi used showed no pronounced effects of exposure to the temperatures alone. It is probable that the cultures X rayed at the extreme temperatures were not at the designated temperature throughout the X ray period because of the short pretreatment exposure of 10 minutes.

*Diplodia zeae*, when X rayed at 10° C. resumed growth after several days. After three days' incubation only a trace of mycelial development was noted in each of the cultures. Two days later large colonies had developed in those receiving 70 and 90 minute treatments, but there was little or no further development in the remaining cultures. Five days later the latter showed definite growth.

Of the cultures X rayed at 20° C. only the culture receiving the 70-minute treatment resumed growth at the end of the 10-day observational period.

The fungi used grew as well on X-rayed agar as on the unirradiated in these tests. However, it is possible that the X-ray treatment affected the medium on which the cultures had been growing, thus accounting wholly or in part for growth differences.

*Diplodia zeae* did not develop spores after a 4-day incubation at 28° C., while the remaining three fungi produced fruiting bodies to some extent. Chlamydospores were produced by *Gibberella saubinetii*. The presence of occasional resistant spores in these cultures may have accounted for the growth in the culture receiving the 70-minute treatment at 40°.

#### TREATMENT OF INFECTED CORN SEEDS

Corn seeds infected by *Diplodia zeae* and *Gibberella saubinetii* were used, since these fungi are most susceptible to X rays in pure cultures and are of more economic importance.

X-ray treatments (120 k. v. (peak), 4 ma., 16 cm target distance, 80 minutes, 375 r units per minute, 50° C. temperature) in the dormant stage of infected seeds did not kill the seed-borne fungi as shown by the vegetative growth made on potato-dextrose agar. This dose injured the corn seeds severely. The fungi in pure cultures are more susceptible than those in the dormant host. This is probably on account of the inactive and resistant form of the parasite and the partial absorption of the X rays by the seeds.

Treatments designed to increase the susceptibility of the parasite without increasing that of the host were attempted. Dormant corn seeds were exposed three days to air saturated with water vapor at 30° C. The control of *Diplodia zeae* was not effected by an X-ray dose distinctly injurious to the host. Corn seeds soaked for periods of 15, 30, 60, 120, and 240 minutes were not freed of *D. zeae* by an X-ray treatment sufficient to cause injury to the seed. Increasing the moisture content of the corn seeds to 30 per cent did not markedly increase the susceptibility of the parasite. The degree of infection resulting from *D. zeae* appears to decrease to some extent in X-rayed seed corn, with storage.

#### FIELD TESTS

In order to test further the effects of X rays on seed-borne organisms dormant corn seeds infected with *Diplodia zeae* and *Gibberella saubinetii* were X-rayed and planted immediately in the field. Seeds that had received an organic mercury treatment, as recommended

by Holbert, Reddy, and Koehler (3), were also planted in order to compare the effects of the two methods of treatment.

Forty kernels were used in each experiment and were planted 1 foot apart in the rows. Four plantings were made at weekly intervals. Infected seedlings were identified by examination of the roots and mesocotyls.

Control of the seed-borne diseases was not effected by the range of treatments used. Total doses of from 375 to 12,000 r units showed no effect except a definite decrease in plant growth when the larger doses were used. X rays having a range of wave lengths obtained by voltages of 56, 78, 120, and 120 k. v. filtered through 1 mm of copper had no effect on the percentage of infection. Intermittent treatments given at weekly intervals did not add to the preventive action. The organic mercury treatment decreased *Diplodia zeae* infection approximately 50 per cent but had no effect on *Gibberella saubinetii*. There was no evidence of stimulation in any of the treatments. The diseased plants were distinctly inferior to those produced by the nearly disease-free corn.

#### TREATMENT OF INFECTED CEREAL SEEDS

Wheat seeds infected with *Ustilago tritici* were X rayed (107 k. v. (peak), 4 ma., 16 cm target distance, 370 r units each minute, seeds 1 cm deep) in the dormant and germinating stages. A treatment of 30 minutes in the dormant stage reduced the percentage of infection to approximately one-third that of the untreated seeds and noticeably injured plant growth.

Germinating wheat seeds (soaked 12 hours, aerated 12 hours) were X rayed 5 minutes (375 r units each minute). Complete control of the fungus resulted, but the plants received serious injury. A 2½-minute treatment decreased infection approximately 50 per cent and also injured the plants. An equivalent treatment, a ¼-mm copper filter being used, reduced infection to approximately one-eighth that of the check without serious injury to the plants.

Dormant wheat seeds infected with *Tilletia levis* were treated similarly. Infected heads were observed in the plants that had received the 40-minute treatment. The percentage of infection of the untreated lot was too small to determine the extent of control.

An X-ray treatment (140 k. v. peak), 4 ma, 24 cm target distance, 240 r units each minute, seeds 1 cm deep) of dormant barley seeds for 68 minutes slightly reduced the infection of *Ustilago nuda* and noticeably reduced the stand and vigor of the plants. A 16-minute treatment of germinating barley seeds (soaked 8 hours, aerated 12 hours) effected complete control but reduced the stand to less than 5 per cent that of the untreated seeds.

X-ray treatments applied to oats in the germinating stage were more effective in the control of *Ustilago avenae* than were those applied to the dormant oat seeds. Doses (140 k.v. (peak), 4 ma., 24 cm target distance, 240 r units each minute, seeds 1 cm deep) applied for 17 minutes to dormant seeds reduced infection approximately 50 per cent. An X-ray treatment of 68 minutes did not reduce infection further. Infection was reduced approximately 25 per cent in the oats treated in the germinating stage (soaked 4 hours, aerated 4 hours) for 33 minutes, but the mature oat plants were slightly injured. This suggests that some of the organisms are more susceptible to X rays.

These treatments indicate that the differentials used in the killing points of the hosts and parasites are not large enough to allow control without serious injury to the hosts. Germinating the seeds affected the differential favorably. The complete control effected in the case of loose smut of barley suggests the use of X rays in investigations where chemical residues are not desired, and where a fraction of the original host population is sufficient.

Negative results secured by other workers, where comparisons could be made, may be ascribed to too short a period of treatment with X rays.

#### SUMMARY

The control of seed-borne diseases by X rays depends on the differential in the killing points of the hosts and their parasites. This differential was found not to be sufficient to permit the complete control of the organisms studied.

While *Diplodia zeae* was quite susceptible to X rays in the actively growing cultures, it was extremely resistant in the dormant corn seed. This probably is due to the inactive condition of the organism, the presence of resistant spores, and the absorption of radiant energy by the seeds. An X-ray dose several times the lethal dose for corn was required to affect the organism.

The decreasing order of tolerance of the fungi used in culture was: *Fusarium moniliforme*, *Cephalosporium acremonium*, *Gibberella saubinetii*, and *Diplodia zeae*. The possible presence of spores in all but *D. zeae* at the time the fungi were X rayed may account for their greater resistance.

Supplementary treatments used in an attempt to modify the fungus or host in order to obtain a more favorable difference in killing points were not successful. The treatments affected the host and parasite similarly.

The organic mercury treatment was effective in partially controlling *Diplodia zeae* but appeared to be ineffective with *Gibberella saubinetii*.

X-ray treatments of dormant wheat, barley, and oat seeds infected with *Ustilago tritici*, *Tilletia levis*, *Ustilago nuda*, and *U. avenae*, respectively, did not effect control.

X-ray treatments of germinating seeds significantly decreased the percentage of loose smut of oats and completely controlled loose smut of barley, although germination was decreased to as low as 5 per cent in the barley.

Seeds as well as fungi vary widely in their lethal X-ray doses. It is possible that seeds requiring large lethal doses may be infected by fungi requiring small lethal doses, in which cases control by X rays may be effective.

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# EFFECT OF LONG-CONTINUED MUSCULAR EXERCISE UPON THE CHEMICAL COMPOSITION OF THE MUSCLES AND OTHER TISSUES OF BEEF CATTLE<sup>1</sup>

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## INTRODUCTION

The quality and palatability of meat are dependent to a great extent upon the chemical and physical properties of the lean, which consists largely of muscle tissue. Among the factors affecting the quality of lean, therefore, muscular exercise may very well be of outstanding importance. Attempts to modify the composition of lean meat by varying the ration fed to the animal have not been successful, since it is evident that cellular protoplasm is capable of maintaining its composition in the face of a varying food supply. But variations in the intensity of functioning of muscle are known to occasion variations in the content of certain of its constituents.

Many investigations have been concerned with a study of the effect of contraction upon the composition of muscle, and much light, especially in recent years, has been thrown upon the problems of muscular metabolism and of the origin of muscular energy. But the ephemeral changes associated with contraction, relaxation, and recovery are not of direct importance to the question of the differences in the composition of meat in an animal accustomed to muscular exercise and in one unaccustomed to muscular exercise. The effects of muscular training on the composition of muscle tissue may be expected to be of a much more permanent nature.

## REVIEW OF LITERATURE

Rogozinski (24)<sup>3</sup> found that the muscles of a dog that had been worked for 12 days contained less water and less fat than did those of a dog that had not been worked. However, the data obtained in an experiment with another pair of dogs did not support the conclusion that long-continued work causes a withdrawal of water. Gerhartz (12) experimenting with two pairs of dogs, confirmed the conclusion that muscular work decreases the water content of muscles. On the other hand, Embden and Habs (8) and Procter and Best (22) found no such effect of training.

The most consistent outcome of experiments concerned with the effect of long-continued muscular work on the chemical composition of muscle relates to the content of glycogen. Although the immediate effect of muscular contraction is to lower the glycogen stores, even

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<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 939.

to almost complete exhaustion in the case of protracted work, the repeated performance of muscular work has been found, in many experiments, to increase markedly the glycogen content of muscle, as much as two or three times the normal value, according to Embden and Habs (8), who used rabbits trained for periods of 2 to 6 weeks. The high content of glycogen in the trained muscles disappears very gradually after the training period is terminated. Procter and Best (22) in their experiments on dogs found that training periods of 7 to 16 days induced a marked increase in glycogen storage in muscles, but that longer periods of training, 20 to 42 days, produced no distinct effect. These results suggested to the authors that there is an optimum period of training for the production of increased glycogen stores and hence that these induced excessive stores are not an essential concomitant of increased ability to perform muscular exercise.

The effect of muscular training on the creatine content of muscle has not been so clearly determined. The most comprehensive of recent investigations concerned with this question are those of Palladin and Ferdmann (21), who experimented with rabbits. Muscular stimulation was brought about by an induction current, applied over the biceps femoris of one leg, the biceps femoris of the other leg serving as a control. In the first experiment, two rabbits were killed after a 5-minute period of stimulation, and the two biceps femoris analyzed. In both rabbits, the stimulated muscle was greatly impoverished in glycogen and contained approximately 4.5 per cent more water than did the unstimulated. The percentage of creatine on the fresh basis was lower in the stimulated muscle than in its control, but expressed either on the dry basis or as a percentage of the total nitrogen content, the percentage of creatine was very nearly the same in stimulated and unstimulated muscle. An entirely different result was obtained, however, when repeated stimulation to induce a condition of muscular training was resorted to. When a 3-minute stimulation was repeated every 4 hours for periods of 5 to 15 days and when the rabbits were sacrificed for chemical examination 48 hours after the last stimulation, the creatine content of the trained biceps femoris was markedly increased over that of the untrained in eight experimental animals. The glycogen content was increased also, while in two of the rabbits on which determinations are reported, there was no difference in moisture content. One or two days training was not sufficient to bring about these results, and it was also shown, in other rabbits, that the effect on the creatine content of the muscle had largely disappeared in 2 or 3 days and entirely in 6 days. The increased glycogen content was plainly evident after six days.

In apparent contradiction to these excellent and consistent experiments of Palladin and Ferdmann may be cited the earlier negative experiments of Mellanby (18) on isolated frog muscle and rabbit muscle *in situ*, and of Brown and Cathcart (5) on decerebrated frogs and later on rabbits. In fact, in the latter case stimulation appeared to have depressed the creatine content of muscle. However, these experiments did not involve the training of muscle, but rather short-time stimulation. In the experiments of Embden and Habs (8) no change in the creatine content of the trained muscle was observed in two rabbits, while in two other rabbits what appeared to be a significant depression in creatine content was found. On the other hand, Ferdmann and Feinschmidt (9), using much the same pro-

cedures as Palladin and Ferdmann and the same species of animal, were able to show that, under their conditions, training increased the creatine-phosphoric acid content of muscle and that this increase disappeared in the course of six days of muscular rest. In these experiments the hexose-phosphoric acid and the pyrophosphoric acid of muscle were not affected by muscular training.

These investigations on the effect of muscular work upon the composition of muscle indicate (1) that the first effects of work are related to the reactions occurring during contraction and recovery, from which the energy is derived; (2) that continued work, or muscular training, produces changes in composition, such as an increase in the content of glycogen and of creatine, either the direct reverse of those related to the contraction phase, or unrelated to them; and (3) that protracted training may cause the first effects of training to disappear gradually and even may cause contrary effects to appear, such as a decrease in creatine content.

The experiments reported in this paper relate to the effects of long-continued muscular training.

#### PLAN OF THE EXPERIMENTS

The experiments extended over two years, from February, 1929, to May, 1931. In each experiment, the subjects were eight steer calves of predominantly Hereford blood, obtained from the same locality and of the same breeding and previous treatment. They weighed initially from slightly less than 500 pounds to slightly more than 600 pounds, and in each experiment they were fairly uniform, especially in the second year's work. The experimental treatment continued in the first experiment from February 13 to June 15, a period of 122 days, and in the second from January 7 to May 18, a period of 131 days.

In the first experiment,<sup>\*</sup> the eight steers were divided into two lots of four each, one lot being exercised daily, the other lot being closely confined. The exercised lot were worked individually for one hour each day on a treadmill set at a slight angle, and the work continued up to the day before slaughter. Each steer walked the equivalent of 450 miles on the horizontal plane, an average of 3.6 miles per day. The exercised steers were full fed a ration of shelled corn, cottonseed meal, corn silage, and alfalfa hay. The unexercised lot were fed the same ration, but in amounts limited so that their gains and conditions would approximate those of the exercised steers. These steers were confined in a small pen in a feeding shed open to the east. The exercised steers also were kept in this pen except during the exercise period, when they were removed to a treadmill next to the pen.

The slaughter weights of the exercised steers averaged 833 pounds, and those of the unexercised steers, 826 pounds. The average daily gains in the two lots were, respectively, 2.33 and 2.56 pounds.

At the time of slaughter, samples of blood for examination and analysis were taken from each steer. Also a special sample of muscle tissue was taken from the upper and inner round for immediate analysis. The livers and hearts (trimmed) were also taken for analysis. From the chilled carcasses, the total lean from each of the nine wholesale cuts from one-half of the carcass, separated from all overlying fat by knife, was ground and sampled for chemical analysis.

<sup>\*</sup>For reasons explained below, this experiment is hereafter designated as experiment 2.

The plan of the second experiment <sup>5</sup> was the same as that of the first, with three exceptions. The steer calves were paired as to initial weight and grade, one calf of each pair being exercised, and during the experimental period the weights of each pair were kept approximately the same by restricting the food consumption of the unexercised calf, the other calf having unrestricted access to food. The ration fed was the same as in the preceding experiment, but the exercise imposed was much greater. Starting at one hour's exercise daily, the period of work was increased to approximately three and a half hours daily. Each exercised steer walked the equivalent of 1,181 miles in 381 working hours, amounting to 8.8 miles per day at a rate of 3.1 miles per hour. As in the preceding experiment, the work was continued up to the day of slaughter. At the time of slaughter, the same samples were taken as in the first experiment, but the selection of lean samples from the chilled carcasses was different, being confined to certain selected muscles from a number of the wholesale cuts of one-half of each carcass. The muscles and the cuts from which they were taken are as follows:

(1) From the round, taken from the rump to the stifle joint, the following muscles were separated: The semimembranosus and adductor muscles, the semitendinosus muscle, and the biceps femoris muscle.

(2) From the short loin, the longissimus dorsi and psoas major muscles were taken.

(3) From the sixth to the twelfth ribs, inclusive, the longissimus dorsi was removed.

(4) From the flank cut, the obliquus externus abdominis was taken.

(5) From the chuck, the triceps brachii muscle was removed from the head of the humerus to the elbow.

The slaughter weights of the unexercised steers in the second experiment averaged 751 pounds, and those of the exercised steers 729 pounds. The average daily gains were, respectively, 1.70 and 1.57 pounds.

In the samples taken, moisture was determined by drying in an electric oven at 105° C. for 5 hours, fat by continuous extraction of the dried samples with ether for 48 hours, nitrogen by the Kjeldahl method, using mercury and potassium sulphate in the digestion mixture and distilling into 4 per cent boric acid, and ash by ignition in an electric muffle furnace at temperatures below 700°. Iron was determined by a slight modification of the Kennedy (14) method, hemoglobin by a modification of Whipple's method (31), and creatine by the procedure developed by Rose, Helmer, and Chanutin (25). The collagen and elastin determinations were made by a method developed in this laboratory (19).

## RESULTS OF THE EXPERIMENT

Although the experiment involving the greater degree of muscular exercise was performed after the experiment involving the smaller degree, it seems advisable to consider it first in the discussion of the results obtained, because the effects of training would be the more pronounced and the more easily discerned. Therefore, in the following discussion it will be referred to as experiment 1.

<sup>5</sup> For reasons explained below, this experiment is hereafter designated as experiment 1.

## THE BLOOD

The hemoglobin in the blood was determined by the Newcomer acid hematin method (20), using a disk that had been standardized by the oxygen capacity method of Van Slyke and Stadie (28). The hemoglobin was determined in a fresh sample of blood, all other constituents in oxalated samples. The results of the determinations are summarized for both experiments in Table 1. There appears to be no effect of exercise on either the water content, the erythrocyte count, the hemoglobin content, or the iron content.

TABLE 1.—*Results of the examination and analysis of the blood samples from steers on heavy and on light work, and from unexercised steers*

Experiment 1, heavy work					Experiment 2, light work			
Treatment and steer No.	Water content	Red cell count	Hemo-globin	Iron content	Treatment and steer No.	Water content	Red cell count	Hemo-globin
		Mil-lions per mm <sup>3</sup>	Grams per 100 cc	Per cent			Mil-lions per mm <sup>3</sup>	Grams per 100 cc
Exercised:	Per cent				Exercised:	Per cent		
84.....	79.68	9.47	11.8	0.0413	80.....	79.12	7.73	13.7
9.....	81.12	8.55	10.6	.0349	116.....	80.41	6.06	12.5
62.....	80.26	10.58	11.6	.0433	119.....	78.77	7.22	14.3
34.....	79.72	8.95	12.2	.0457	125.....	78.93	10.60	13.1
Average.....	80.20	9.39	11.6	.0412	Average.....	79.31	7.90	13.4
Unexercised:					Unexercised:			
23.....	80.97	12.17	11.5	.0370	103.....	80.09	4.92	12.0
21.....	81.34	10.17	10.7	.0372	117.....	78.84	8.78	13.7
43.....	79.45	10.22	11.5	.0455	121.....	78.42	9.49	14.3
36.....	81.44	9.30	10.3	.0413	129.....	77.99	10.08	14.3
Average.....	80.80	10.47	11.0	.0402	Average.....	78.84	8.32	13.6

In both experiments, but particularly in experiment 1, the red cell counts are higher than those usually found for cattle blood. Thus, Marloff (17) has reported an average of 6.51 millions per cubic millimeter; Kuhl (15), 5.72; Wells and Sutton (30), 7.65; Schenkenbach (26), 8.05 for males and 7.47 for females; Bonnier, Jorpes, and Sköld (4), 5.46; Drastich (7), 6.41; and Hayden and Fish (13), 6.55.

The hemoglobin values in experiment 1, averaging 11.3 g per 100 cc, are approximately the same as the average of 11.7 reported by Marloff (17), 10.8 by Kuhl (15), 10.55 by Drastich (7), and 10.9 and 11.29 by McCay (16) in two large series of analyses of the blood of dairy cows. The latter investigators report an average of  $12.2 \pm 0.8$  g of hemoglobin per 100 cc of blood for six mature bulls of four different breeds. The hemoglobin values in experiment 2, averaging 13.5 g per 100 cc of blood, although carried out by the same technic, were substantially higher.

The iron content of the blood, determined in experiment 1, averaged 0.0407 per cent. This value is somewhat higher than the average value obtained by McCay (16) of 38.9 mg of iron per 100 cc of blood, from 226 analyses, and the average value of 36.5 mg per 100 cc of blood reported by Awdejewa and his associates (1), from observations on nine cows. Wagenaar (29) obtained an average of 0.042 per cent of iron in the blood of four cows.

## THE IRON AND HEMOGLOBIN CONTENTS OF THE TISSUES

Hemoglobin determinations were made upon the heart and the special sample from the round in experiment 1 (heavy work), and upon the liver, round, ribs, and shank samples in addition in experiment 2. The results of these analyses, expressed in percentage of the fresh tissue, are summarized in Table 2. They are of interest, in view of the scarcity of such information in the literature, even though they do not demonstrate any effect of muscular training. In experiment 2 (light work) the averages for five of the six samples are higher for the exercised than for the unexercised steers. However, the variations among the steers within each group were so great that average group differences are obviously insignificant.

TABLE 2.—Hemoglobin content (per cent) of the tissues from steers on heavy and on light work, and from unexercised steers

Experiment 1, heavy work			Experiment 2, light work						
Treatment and steer No.	Hemoglobin in—		Treatment and steer No.	Hemoglobin in—					
	Heart	Special sample of round		Liver	Heart	Special sample of round	Round	Ribs	Shank
Exercised:			Exercised:						
84.....	0.404	0.283	80.....	0.328	0.286	0.295	0.258	0.198	0.258
9.....	.382	.387	116.....	.352	.348	.225	.195	.196	.232
62.....	.325	.354	119.....	.405	.412	.211	.171	.162	.239
34.....	.463	.389	125.....	.297	.361	.260	.194	.174	.336
Average.....	.394	.353	Average.....	.346	.352	.248	.205	.183	.266
Unexercised:			Unexercised:						
23.....	.393	.388	103.....	.418	.338	.190	.170	.189	.257
21.....	.448	.370	117.....	.290	.325	.242	.208	.184	.210
43.....	.354	.346	121.....	.393	.292	.280	.181	.192	.192
36.....	.451	.361	129.....	.164	.264	.308	.170	.161	.253
Average.....	.412	.366	Average.....	.316	.305	.255	.182	.182	.228

In experiment 1 (heavy work) all of the eight samples of muscles taken from the chilled carcasses were analyzed for iron, making a total of 64 analyses. The results revealed no appreciable differences between cuts and no significant differences between exercised and unexercised groups. The average result was  $0.003075 \pm 0.000042$  per cent of iron in the fresh tissues.

In experiment 2 (light work) iron analyses were made upon the marrow from one of the femur bones of each of the carcasses. On the same samples, analyses were made also for dry substance and fat. The results in Table 3 show that, in the group of exercised steers, the marrow contained 20 per cent less iron than did that of the unexercised group. By applying Fisher's modification of Student's method (11, p. 107) to these two groups of results, a value of  $t=4.83$  is obtained, and the probability that a difference as great as that observed (0.00052) would result from chance is considerably less than 0.01. This lowering of the iron content of the marrow was associated with an increased content of dry matter, but essentially no difference in fat content. There was an average of 4.14 per cent of dry matter not fat in the marrow samples from the exercised steers and an

average of only 1.94 per cent of dry matter not fat in the marrow samples from the unexercised steers.

TABLE 3.—*Chemical composition (per cent) of the marrow from the femur bones from steers on light work (experiment 2) and from unexercised steers*

Treatment and steer No.	Dry substance	Ether extract	Iron	Treatment and steer No.	Dry substance	Ether extract	Iron
Exercised:				Unexercised:			
80.....	97.43	95.23	0.00197	103.....	96.79	97.53	0.00237
116.....	97.88	94.62	.00216	117.....	96.71	93.58	.00282
119.....	95.58	89.32	.00206	121.....	91.24	89.45	.00256
125.....	97.41	92.58	.00214	129.....	95.21	91.63	.00266
Average.....	97.08	92.94	.00208	Average.....	94.99	93.05	.00260

#### THE FAT AND WATER CONTENTS OF THE TISSUES

The results of the analysis of the tissue samples for fat and water are given in Table 4. The fat percentages are on the fresh basis, but the water percentages are computed on the protoplasmic (fat-free) basis, in order to eliminate the considerable influence of variations in fat content.

TABLE 4.—*Fat and water content of tissue samples from steers on heavy and on light work, and from unexercised steers*

#### EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF FAT ON THE FRESH BASIS

Treatment and steer No.	Heart	Liver	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
				Semi-membranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
Exercised:											
84.....	2.38	1.67	1.06	0.80	1.26	1.30	1.19	2.67	1.78	3.48	1.24
9.....	2.86	1.54	1.73	2.37	2.00	1.46	2.78	4.22	3.06	5.20	2.34
62.....	2.97	1.63	1.13	1.01	1.24	2.32	.96	2.26	1.68	2.06	1.97
34.....	2.88	1.56	1.28	1.38	1.68	1.39	2.20	3.08	2.14	2.77	1.83
Average.....	2.77	1.60	1.30	1.39	1.54	1.62	1.78	3.06	2.16	3.38	1.84
Unexercised:											
23.....	3.20	2.36	1.53	1.39	1.27	2.14	2.33	4.50	1.98	2.90	3.39
21.....	3.62	2.00	1.52	1.54	2.00	1.40	1.58	3.16	2.07	3.46	2.18
43.....	2.56	2.39	1.62	2.50	1.40	1.86	1.49	2.61	2.52	2.49	1.95
36.....	3.63	2.90	1.60	1.65	1.75	2.25	1.98	3.15	2.05	2.67	2.60
Average.....	3.25	2.41	1.57	1.77	1.60	1.91	1.84	3.36	2.16	2.88	2.53

#### EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF WATER ON THE PROTOPLASMIC BASIS

Exercised:											
84.....	80.60	71.01	75.73	76.13	76.07	76.40	75.85	77.54	76.91	76.86	76.54
9.....	80.21	71.52	75.71	75.65	76.20	76.20	76.32	76.99	76.34	75.79	76.51
62.....	81.08	71.20	76.31	76.13	76.39	76.48	75.92	77.95	76.88	76.49	77.32
34.....	81.11	70.94	76.60	76.11	76.05	76.57	76.23	77.87	76.79	76.08	77.58
Average.....	80.75	71.17	76.09	76.01	76.18	76.41	76.08	77.59	76.73	76.30	76.99
Unexercised:											
23.....	81.63	72.27	76.93	76.48	76.19	76.83	76.43	78.39	77.00	77.91	78.40
21.....	81.56	72.36	77.23	76.66	76.98	77.25	76.44	78.33	77.00	77.69	77.71
43.....	82.18	73.54	77.25	77.74	76.97	76.54	76.68	78.14	78.16	76.65	77.51
36.....	81.12	74.26	76.44	76.23	76.37	77.30	76.07	77.86	76.53	77.33	77.20
Average.....	81.62	73.11	76.96	76.78	76.63	76.98	76.40	78.18	77.17	77.39	77.70

TABLE 4.—*Fat and water content of tissue samples from steers on heavy and on light work, and from unexercised steers*

## EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF FAT ON THE FRESH BASIS

Treatment and steer No.	Heart	Liver	Special sample of round	Round	Ribs	Fore shank	Loin end	Short loin	Rump	Plate	Chuck	Flank
Exercised:												
80.....	1.58	1.54	2.34	4.10	9.47	5.87	7.20	8.05	6.51	13.49	6.81	12.27
116.....	1.86	1.36	2.58	4.60	8.38	4.45	5.28	6.60	6.52	14.15	7.35	11.88
119.....	1.97	2.05	3.40	4.71	9.00	5.02	6.51	8.90	7.05	13.80	7.45	10.21
125.....	2.19	1.92	3.02	3.51	7.77	4.38	5.31	7.04	5.78	12.24	6.67	8.21
Average.....	1.90	1.72	2.84	4.23	8.66	4.93	6.08	7.65	6.47	13.42	7.07	10.64
Unexercised:												
103.....	( <sup>a</sup> )	2.68	4.13	5.14	12.13	4.67	8.06	10.46	9.43	14.93	8.69	( <sup>b</sup> )
117.....	2.55	2.72	2.34	4.37	8.94	5.15	5.31	8.26	5.29	13.35	6.93	9.80
121.....	2.17	2.54	2.51	2.20	8.81	3.99	6.19	7.07	5.29	10.54	6.99	9.94
129.....	2.14	2.57	2.27	3.62	9.45	5.96	5.28	6.82	6.47	12.96	7.23	8.00
Average.....	2.38	2.63	2.81	3.83	9.83	4.94	6.21	8.15	6.62	12.95	7.46	9.37

## EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF WATER ON THE PROTOPLASMIC BASIS

Exercised:												
80.....	79.99	70.44	75.34	75.79	76.26	75.42	76.26	76.14	75.57	75.93	76.55	75.17
116.....	81.34	71.10	75.85	76.51	75.81	77.26	76.82	77.08	76.80	77.11	77.24	76.06
119.....	80.86	71.64	75.86	76.35	75.52	75.84	76.76	76.38	76.22	76.68	77.19	75.27
125.....	81.54	72.69	75.93	75.97	75.39	76.53	76.51	75.60	76.46	76.14	76.32	75.06
Average.....	80.93	71.47	75.74	76.15	75.74	76.26	76.59	76.30	76.21	76.46	76.82	75.39
Unexercised:												
103.....	81.85	73.62	76.25	76.13	77.28	77.24	77.49	76.67	76.32	76.11	77.09	( <sup>b</sup> )
117.....	80.99	73.55	75.34	76.97	77.26	76.59	76.82	76.35	77.53	77.03	77.09	75.85
121.....	81.04	74.31	76.07	76.26	76.68	76.61	76.27	77.67	76.04	76.07	76.70	75.69
129.....	81.23	73.27	75.05	75.67	76.27	75.94	76.15	75.86	76.56	76.47	76.73	75.05
Average.....	81.28	73.69	75.68	76.26	76.87	76.59	76.68	76.64	76.61	76.42	76.90	75.53

<sup>a</sup> The excess fat was not trimmed from this sample before analysis.<sup>b</sup> This sample was lost.

In experiment 1 there is a general tendency for the tissues from the exercised steers to exhibit a smaller fat content than the corresponding tissues from the unexercised steers. Of the 11 tissues examined, only 2, the longissimus dorsi muscle of the rib cut and the obliquus externus abdominis of the flank cut, failed to show this relation between the averages of the two groups of steers.

The most pronounced difference in fat content occurred in the case of the liver samples. Here the average difference amounts to 0.81 per cent of fat; the value of *t* (essentially the ratio of the average difference to the standard deviation of percentages from their respective group mean) is 4.33, and the probability that as marked a difference as this could have been the result of chance is considerably less than 0.01. Thus, even with these small groups of values, a clear demonstration of an effect of muscular training in reducing the concentration of ether-soluble substances in the liver was obtained.

However, no other tissue showed a difference between exercised and unexercised steers sufficiently marked, in relation to the variation occurring within each group, to indicate that muscular training had produced it. The heart tissue, the special sample from the round, and the chuck muscle come the nearest to a demonstration, but the



probabilities that chance may have produced the average differences are so large (0.15, 0.13, and 0.13, respectively) that they can not be neglected. Even if all the muscle samples are considered together in two groups of 36 analyses each, and the deviations are computed from the averages of each group of four samples in arriving at the standard deviation, the probability obtained is greater than 0.10, and hence the possibility that chance was responsible for group differences can not be neglected.

In experiment 2, involving light work, the fat content of the livers of the exercised steers is again significantly lower than that of the unexercised steers, the average difference amounting to 0.91 per cent of fat. The value of  $t$ , according to Fisher's method, is 5.46, giving a probability for  $n=6$  of considerably less than 0.01 that chance conditions alone brought about this distribution of values into the two groups. It may, therefore, be concluded that the work imposed upon the steers in one of these groups was responsible for lowering the content of ether-soluble constituents in the liver.

The heart samples also suggest strongly an effect of work similar to the effect upon the liver, but the probability in this case amounts to about 0.044 that chance factors may have brought this result about, equivalent to odds of 1 in 24. Any great amount of reliance can not, therefore, be placed on the significance of this comparison.

With respect to the fat contents of the muscle samples in experiment 2, nothing can be said concerning a possible effect of muscular training. Considering group averages only, in eight comparisons the exercised steers gave the lower figure, while in four comparisons the reverse was true.

Table 4 shows that with respect to all tissue samples taken in experiment 1, the water content on the protoplasmic basis averaged less for the exercised steers than for the unexercised steers. The difference was greatest and most distinct for the liver, amounting to 1.94 per cent of water. The value of  $t$  in this case is 3.90, and from Fisher's table (11, p. 139), only one value in a hundred ( $n$  being equal to 6) will exceed 3.707 by chance; hence, the difference is highly significant. It may, therefore, be concluded that continued exercise lowered the water content of the livers of these steers.

For a number of other samples, the differences between the two groups of steers in the percentage of water are significant, the value of  $P$  being less than 0.033, corresponding to odds of 1 in 30 that chance alone would produce the result, that is, heart 0.032, special sample of round 0.026, biceps femoris (round) muscle 0.032, and obliquus externus abdominis (flank) muscle 0.018. If the results on all the muscles are combined in a single statistical analysis, in which the standard deviation is computed from the deviations of each group of four percentages from its mean, the mean difference is 0.0668 per cent and the value of  $t$  becomes 7.67,  $n$  being 70. This value of  $t$  is far beyond the range of Fisher's table, so that it is perfectly clear that, as the statistician would say, the percentages of water in the muscles from the exercised and unexercised steers were not drawn from the same "population." Hence, it may be concluded that, continued muscular exercise of the intensity imposed in this experiment reduces the moisture content of liver, heart, and muscles.

In experiment 2 the percentages of water on the protoplasmic basis were also lower for the exercised steers with the exception of

two samples, the plate and the special sample of round, but the average differences are considerably smaller than those obtained in experiment 1 and, hence, less certainly caused by the difference in muscular activity. With only two samples is the probability that all analyses are from the same population sufficiently small that it may be neglected. For the liver samples, the average difference in water content was 2.22 per cent, the value of  $t$  being 4.24, and the probability considerably less than 0.01. Also for the rib samples the average difference was 1.13 per cent, the value of  $t$  3.63, and the probability approximately 0.011. If all the muscle samples are combined in one analysis, as was done in the discussion of the corresponding results of experiment 1, the mean difference is 0.265 (as compared with 0.668), the value of  $t$  becomes 2.45 (as compared with 7.67), and  $P$  is somewhat less than 0.02. It appears, therefore, that in this experiment also the exercise imposed has markedly lowered the moisture content of liver and also that of the muscles, though to a less pronounced extent, than did the more severe muscular exercise imposed in experiment 1.

The analyses of fat and water in a number of tissues from 16 different steer calves offers an opportunity of studying statistically the relation between the composition of different muscular tissues in the same animal. This relationship is of general interest, and in addition it bears directly upon the interpretation of the data with respect to the effect of continued muscular exercise upon the fat and water content of the muscles, since it will show to what extent the analyses of the different samples are interdependent, and conversely, to what extent each sample analyzed is an independent test of the effect of muscular work. The product-moment correlation method was therefore applied to the water and fat analyses in each experiment, the standard deviations being computed from the deviations of each group of four analyses from its own mean. In computing the deviation products every possible pair of deviations was taken from all the muscle samples of each steer.

In experiment 1 the correlation coefficient for fat content was +0.360, and that for water content +0.182. In experiment 2 the correlation coefficients were, respectively, +0.519 and +0.346. These coefficients measure the degree to which variations in the fat and water content in one sample of muscle tissue are associated with variations in another sample, perfect correlation being measured by a coefficient of 1 and the positive sign indicating that a high content in one muscle tends to be associated with a high content in another. Evidently a closer correlation exists among the different tissues with respect to fat than with respect to water, and also, apparently, there is a closer correlation between the fat content, as well as the water content, of lean samples from the same carcass, freed roughly from visible fat with a knife, than between the fat content or the water content of individual muscles, dissected out in a manner better calculated to avoid contamination with intermuscular fat. The correlation coefficients obtained in the first experiment are inconsiderable, though significant in all probability in indicating the existence of some degree of positive correlation. The coefficients obtained in the second experiment are somewhat larger, though still too small to indicate that the fat or moisture content of one lean sample may be predicted satisfactorily from that of another.

## BOUND WATER IN MUSCLE

In experiment 1 (heavy work) an attempt was made to determine whether trained muscle possessed a different percentage of "bound" water than untrained muscle. The conception of "bound" water followed was that of Rubner, according to which "bound" water is that fraction of the water in the tissue which does not freeze at temperatures as low as  $-20^{\circ}\text{C}$ . The method of determining bound water was essentially that of Robinson (23), which is a modification of the method of Thoenes (27). The sample used for the determination was the special sample from the round, taken immediately after slaughter, and the determinations were made within 48 hours. The results are assembled in Table 5.

TABLE 5.—*Bound-water content of the special samples of round lean from steers on heavy work, experiment 1*

[All results are expressed on the fat-free basis]

Treatment and steer No.	Total water	Bound water			Treatment and steer No.	Total water	Bound water		
		As percentage of total water	As percentage of fat-free substance	Per gram of fat-free dry matter			As percentage of total water	As percentage of fat-free substance	Per gram of fat-free dry matter
Exercised:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Gram</i>	Unexercised:	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Gram</i>
84.....	75.73	14.3	10.8	0.45	23.....	76.93	14.5	11.1	0.48
9.....	75.71	14.6	11.0	.45	21.....	77.23	11.9	9.2	.40
62.....	76.31	12.6	9.6	.41	43.....	77.25	12.1	9.3	.41
34.....	76.60	13.7	10.5	.45	36.....	76.44	13.2	10.1	.43
Average...	76.09	13.8	10.5	.44	Average...	76.96	12.9	9.9	.43

It is clear that no evidence was obtained of an effect of training on the bound-water content of muscle.

## THE NITROGEN CONTENT OF THE FAT-FREE DRY MATTER OF THE TISSUES

The nitrogen content of the tissue samples in both experiments has been expressed on the basis of the fat-free dry matter, in order to eliminate the variations in fat and water, which have been shown to be, to some extent, influenced by muscular training. The percentages of nitrogen thus expressed are presented in Table 6.

In considering experiment 1 involving heavy work, first, it appears that for all tissues examined except two the average percentage of nitrogen for the exercised steers is lower than that for the unexercised. In one case, the semitendinosus muscle of the round, the averages for the two groups of steers are the same, while in another case, the biceps femoris of the round, there is a difference of 0.02 per cent in favor of the exercised steers.

As in all other cases in which training has affected the chemical composition of tissues, the composition of the liver is affected the most. In this instance, the average reduction of the nitrogen content of the fat-free dry matter is 1.33 per cent of nitrogen. Applying Fisher's method of statistical analysis, the standard deviation  $s$  is 0.502,  $t$  of 3.75, and the probability that the two sets of data are from the same population is less than 0.01. There can be little doubt that

muscular training has reduced the nitrogen content of the fat-free dry matter of the liver.

TABLE 6.—*Nitrogen content (per cent) of the fat-free dry matter of the tissues from steers on heavy and on light work, and from unexercised steers*

EXPERIMENT 1, HEAVY WORK

Treatment and steer No.	Heart	Liver	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
				Semi-membranosus	Semiten-dinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Tri-ceps brachii
Exercised:											
84.....	14.68	11.12	14.91	14.70	14.90	14.86	14.46	16.01	14.90	15.00	14.50
9.....	14.67	11.59	15.17	14.72	15.48	15.65	15.25	15.15	15.08	15.34	14.82
62.....	14.98	11.26	15.20	15.19	15.69	15.04	14.88	15.36	15.27	14.81	15.07
34.....	15.42	11.18	15.24	15.03	15.63	15.32	14.84	15.62	15.24	15.26	14.99
Average.....	14.94	11.29	15.13	14.91	15.43	15.22	14.86	15.54	15.12	15.10	14.85
Unexercised:											
23.....	14.90	11.96	15.40	15.14	14.80	14.91	14.68	16.81	15.44	15.85	14.95
21.....	15.19	12.29	15.79	15.14	15.34	15.47	15.01	15.44	15.36	15.41	15.50
43.....	15.09	12.70	15.64	16.18	15.76	14.94	15.02	15.27	15.83	15.11	14.78
36.....	15.50	13.53	15.27	15.10	15.81	15.46	15.17	14.93	15.27	15.78	15.35
Average.....	15.17	12.62	15.53	15.39	15.43	15.20	14.97	15.61	15.48	15.54	15.15

EXPERIMENT 2, LIGHT WORK

Treatment and steer No.	Heart	Liver	Special sample of round	Round	Ribs	Fore shank	Loin end	Short loin	Rump	Plate	Chuck	Flank
Exercised:												
80.....	14.27	11.10	14.70	15.03	15.50	15.64	15.12	15.54	15.11	15.23	15.70	15.98
116.....	15.02	11.72	15.51	14.99	14.76	16.06	15.35	15.51	16.14	16.32	15.36	15.73
119.....	15.72	12.17	15.18	14.82	14.59	14.77	16.38	15.94	15.11	15.77	15.63	15.31
125.....	16.17	11.98	15.12	15.01	14.58	16.09	15.51	15.17	15.73	15.71	15.38	16.34
Average.....	15.30	11.74	15.13	14.96	14.86	15.64	15.59	15.54	15.52	15.51	15.52	15.84
Unexercised:												
103.....		12.82	15.25	14.71	15.22	16.18	15.41	15.37	15.06	15.01	15.01	-----
117.....	14.52	13.18	14.41	15.35	15.60	15.72	15.90	15.81	15.88	15.33	15.53	15.29
121.....	14.99	13.30	15.60	14.81	15.00	15.32	15.14	16.24	15.20	15.27	15.14	15.71
129.....	14.36	13.17	14.89	14.29	15.26	15.64	15.23	15.38	15.56	15.63	15.28	15.03
Average.....	14.62	13.12	15.04	14.79	15.27	15.72	15.42	15.70	15.43	15.31	15.24	15.34

In the special sample of tissue from the round, there was an average difference of 0.40 per cent of nitrogen, a standard deviation of 0.196, a value of  $t$  is 2.88, and a probability of about 0.032 that the two sets of data are the result of the operation of the same factors. The indications are clear with this tissue also, muscular training has lowered the nitrogen content of the fat-free dry matter. The other muscle samples, secured from the chilled carcasses, do not individually show clear evidence of such an effect of training; the longissimus dorsi muscle of the ribs gives the most significant evidence. but the probability that the two groups of analyses may have been produced by

the same factors is too large (0.054) to be safely neglected. However, if all of these eight muscle samples are considered together in the same statistical analysis, computing the standard deviation from the deviations of each group of four analyses from its own mean, an average difference of 0.217 per cent is obtained between exercised and unexercised steers, the standard deviation is 0.334,  $t$  is 2.59, and the probability that the same factors operated in determining the percentage for both groups of steers is less than 0.02. Such a low probability of a chance outcome may be neglected, and it may be concluded that, in muscle tissue generally, the effect of muscular training is to lower the nitrogen content of the fat-free dry matter.

In experiment 2 (light work) the liver undoubtedly has suffered the same large reduction (1.38 per cent) in the nitrogen content of the fat-free dry matter. The value of  $t$  in this comparison is 5.40, and the probability is considerably less than 0.01. But the heart and the special round sample show no such relation, and of the nine muscle samples from the chilled carcasses only three show an average reduction in the percentage of nitrogen for the exercised steers. Evidently the intensity of the work imposed in this experiment was not sufficient to affect appreciably the nitrogen content of the fat-free dry matter.

#### THE GLYCOGEN CONTENT OF THE FRESH MUSCLE

In accounting for the reduction in the nitrogen content of the fat-free dry matter of the tissues brought about by muscular training and especially evident in the results of experiment 1, one naturally, perhaps, thinks first of an increase in glycogen. But it would require an improbably large increase in the glycogen content of the tissues to account for the decreases in nitrogen observed. In experiment 1, in which the average nitrogen content of the liver on the fat-free dry-matter basis decreased from 12.62 to 11.29 per cent, it would require an increase of glycogen equal to 10.54 per cent of the dry matter. In experiment 2, the average reduction in the nitrogen percentage in the liver was from 13.12 to 11.74, which would again call for an increase in glycogen equal to 10.52 per cent of the dry matter. In the case of the fresh round sample in experiment 1, the nitrogen content decreased on an average from 15.53 per cent of the fat-free dry matter to 15.13 per cent. If this had been brought about by an increased deposition of glycogen, the increase would have to equal 2.58 per cent of the fat-free dry matter.

Glycogen determinations were made upon the fresh samples of the round taken in experiment 1, using the usual method of alkali decomposition of the tissues and alcohol precipitation of the glycogen. The special technic recommended by Fisher and Lackey (10) was followed in these analyses. The results for exercised steers, in the order in which they have been arranged in the tables, are 3.92, 3.10, 3.63, and 3.73 per cent of glycogen on the fat-free dry matter basis, averaging 3.60 per cent. For the unexercised steers the results are, in the order given in the tables, 2.96, 1.48, 2.45, and 3.28 per cent of glycogen, averaging 2.54 per cent. These values indicate that muscular training may have increased the glycogen content of the dry muscle. Statistical analysis gives a probability of 0.05 that the two series of analyses resulted from the same combination of factors, so that the above interpretation can not be said to have been demonstrated.

## THE LIPOID CONSTITUENTS OF THE TISSUES

Since an accumulation of glycogen in the tissues of the trained animals can not reasonably account in full for the reduction in the nitrogen content of the fat-free dry matter, another possibility is that there is an increased content of lipoids in the tissues of the trained animals. It is known that ether will not extract all the lipoids from tissues. Although none of the data obtained in this investigation can throw any direct light upon this question, reference may be made, in support of the explanation offered above, to the work of Bloor (3), who, in investigations of the lipid content of a few selected beef muscles, found that the contents of cephalin and lecithin varied from muscle to muscle in the order of what might be considered to be their activity, the more active the muscle the higher its percentage content of phospholipid. Furthermore, Cowdry (6) in a recent discussion of the mitochondrial constituents of protoplasm (in all probability largely phospholipid in character), has noted the association of mitochondria with intense protoplasmic activity. He says (6, p. 82):

In cytomorphosis, for example, they are especially numerous in the active stages in the life of the cell and they diminish with senility in both plants and animals. There is a sharp increase in mitochondria with regenerative activity, in compensatory hypertrophy, and in many other conditions.

Of particular interest to the interpretation of the results of these experiments are the following observations of Cowdry:

\* \* \* there is a distinct reciprocal relationship between the amount of mitochondria and the amount of fat. Where there are few mitochondria there is much fat, and *vice versa*. Decreased oxidation favors the accumulation of fat and increased oxidation favors its elimination, which suggests at once some connection between the amount of mitochondria and oxidation; and their abundance in the active stages of the life of the cell, where protoplasmic respiration is rapid, points to the same conclusion.

Hence, the decrease in ether-soluble constituents observed in the exercised as compared with the unexercised steers (Table 4) may be considered indirect evidence of an increased phospholipid content.

## CREATINE CONTENT OF THE TISSUES

In experiment 1 creatine was determined in all the tissues. In experiment 2 only six of the samples were thus analyzed. The results obtained have been summarized in Table 7. They are expressed (1), on the protoplasmic basis to eliminate the effect of variations in the fat content of the tissues, (2) on the fat-free and water-free basis, to eliminate the effect of variations in both the fat and the water content of the tissues, and (3) for the data of experiment 1 only the creatine nitrogen is expressed as a percentage of the total nitrogen.

TABLE 7.—*Creatine content of the tissue samples from steers on heavy and on light work, and from unexercised steers*

## EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF CREATINE ON THE PROTOPLASMIC BASIS

Treatment and steer No.	Heart	Liver	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
				Semi-membranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triiceps brachii
Exercised:											
84.....	0.274	0.010	0.440	0.461	0.567	0.466	0.460	0.619	0.469	0.456	0.406
9.....	.232	.020	.380	.402	.546	.484	.420	.627	.424	.399	.427
62.....	.234	.020	.380	.431	.563	.461	.441	.564	.448	.405	.402
34.....	.238	.024	.389	.499	.518	.465	.467	.554	.485	.449	.419
Average.....	.245	.019	.397	.448	.549	.469	.447	.591	.457	.427	.414
Unexercised:											
23.....	.245	.012	.515	.434	.525	.443	.414	.519	.450	.463	.381
21.....	.263	.020	.470	.437	.577	.473	.477	.663	.477	.473	.439
43.....	.263	.044	.428	.461	.586	.469	.437	.533	.426	.416	.444
36.....	.276	.058	.439	.511	.639	.503	.484	.597	.491	.434	.448
Average.....	.262	.034	.463	.461	.582	.472	.453	.578	.461	.447	.428

## EXPERIMENT 1, HEAVY WORK, PERCENTAGE OF CREATINE ON THE FAT-FREE AND WATER-FREE BASIS

Exercised:											
84.....	1.41	0.035	1.81	1.93	2.37	1.97	1.90	2.76	2.03	1.97	1.73
9.....	1.17	.071	1.56	1.65	2.29	2.03	1.77	2.72	1.79	1.65	1.82
62.....	1.24	.071	1.60	1.81	2.38	1.96	1.83	2.56	1.94	1.72	1.77
34.....	1.26	.084	1.66	2.09	2.16	1.98	1.96	2.50	2.09	1.88	1.87
Average.....	1.27	.065	1.66	1.87	2.30	1.99	1.87	2.64	1.96	1.81	1.80
Unexercised:											
23.....	1.33	.044	2.23	1.84	2.20	1.91	1.76	2.40	1.96	2.10	1.76
21.....	1.43	.074	2.06	1.87	2.51	2.08	2.02	3.06	2.07	2.12	1.97
43.....	1.48	.166	1.88	2.07	2.54	2.00	1.87	2.44	1.95	1.78	1.97
36.....	1.46	.224	1.86	2.15	2.70	2.22	2.02	2.70	2.09	1.91	1.96
Average.....	1.42	.127	2.01	1.98	2.49	2.05	1.92	2.65	2.02	1.98	1.92

## EXPERIMENT 1, HEAVY WORK, CREATINE NITROGEN AS A PERCENTAGE OF THE TOTAL NITROGEN

Exercised:											
84.....	3.16	0.101	3.94	4.25	5.16	4.32	4.27	5.67	4.45	4.36	3.87
9.....	2.64	.197	3.37	3.63	4.85	4.23	3.84	6.02	3.93	3.63	4.03
62.....	2.73	.201	3.42	3.85	4.93	4.27	3.98	5.46	4.14	3.81	3.85
34.....	2.70	.240	3.54	4.52	4.51	4.21	4.34	5.30	4.49	4.05	4.07
Average.....	2.81	.185	3.57	4.08	4.86	4.26	4.11	5.61	4.25	3.96	3.96
Unexercised:											
23.....	2.96	.119	4.72	3.96	4.84	4.20	3.93	4.80	4.15	4.37	3.92
21.....	3.12	.193	4.26	4.03	5.35	4.37	4.39	6.56	4.42	4.57	4.16
43.....	3.22	.430	3.92	4.21	5.25	4.37	4.06	5.26	4.05	3.88	4.37
36.....	3.14	.550	3.98	4.64	5.58	4.70	4.36	5.98	4.48	4.00	4.21
Average.....	3.11	.323	4.22	4.21	5.26	4.41	4.19	5.65	4.28	4.21	4.17

## EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF CREATINE ON THE PROTOPLASMIC BASIS

Treatment and steer No.	Liver	Heart	Special sample of round	Round	Ribs	Shank
Exercised:						
80.....	0.041	0.261	0.441	0.411	0.418	0.358
116.....	.041	.228	.434	.392	.287	.291
119.....	.043	.233	.459	.407	.401	.346
125.....	.047	.226	.425	.432	.394	.332
Average.....	.043	.237	.440	.411	.375	.332
Unexercised:						
103.....	.032	.223	.471	.423	.411	.347
117.....	.035	.265	.476	.384	.393	.322
121.....	.053	.288	.487	.413	.406	.371
129.....	.041	.295	.471	.416	.432	.364
Average.....	.040	.268	.476	.409	.411	.351

## EXPERIMENT 2, LIGHT WORK, PERCENTAGE OF CREATINE ON THE FAT-FREE AND WATER-FREE BASIS

Treatment and steer No.	Liver	Heart	Special sample of round	Round	Ribs	Shank
Exercised:						
80.....	0.139	1.30	1.79	1.70	1.76	1.46
116.....	.142	1.22	1.80	1.67	1.19	1.28
119.....	.152	1.22	1.90	1.72	1.64	1.43
125.....	.172	1.22	1.77	1.80	1.60	1.41
Average.....	.151	1.24	1.82	1.72	1.55	1.40
Unexercised:						
103.....	.121	1.23	1.98	1.77	1.81	1.52
117.....	.132	1.39	1.93	1.67	1.73	1.38
121.....	.206	1.52	2.04	1.74	1.74	1.59
129.....	.153	1.57	1.89	1.71	1.82	1.51
Average.....	.153	1.43	1.96	1.72	1.78	1.50

Of the 11 tissue samples analyzed for creatine in experiment 1, 10 showed smaller average percentages of creatine on the protoplasmic basis for the exercised steers than for the unexercised. While this outcome is suggestive of an effect of muscular training it falls short of a demonstration. The average differences are in the main slight, and the application of statistical analysis, either to the analytical results of individual tissues or of all muscular tissues combined, does not give any clear indication that the creatine content of the tissues of the two groups of steer calves was determined by any factors but those common to both groups. It is worthy of note that the creatine percentages for the different muscle samples are all of about the same order of magnitude, except those for the semitendinosus of the round and the psoas major of the short loin, which contain more than 0.1 per cent more creatine.

The percentages of creatine expressed on the fat-free and water-free basis for experiment 1 offer clearer evidence of a depressing effect of muscular training on the creatine content. All tissue samples show a lower average percentage of creatine for the exercised steers. Statistical analysis of the results of individual tissues reveals a decisive result only in the case of the special sample of round. Here the average difference is 0.35, the standard deviation is 0.145,  $t$  is 3.41, and the probability is about 0.015 that the values for the two groups of steers were the result of common factors only. It seems fair to neglect this small probability and to conclude that with respect to this sample some factor operating in one group but not in the other



accounted for the average depression in creatine content. This factor could only have been the muscular work imposed on one of the groups of steers.

A comparison of the creatine percentages of the two groups of steers with respect to heart muscle indicates a probability of slightly less than 0.05 that a random operation of common factors alone determined the difference between groups. While this probability is too large to be neglected, it is highly suggestive that muscular training was involved in the production of the lower percentages for the exercised steers.

A further statistical analysis was made by combining all the analyses of muscle samples that contain approximately the same percentage of creatine, except the special sample from the round which gave significant results by itself. The two muscles with a high creatine content, the semitendinosus and the psoas major, were thus excluded. For the remaining six muscles, the unexercised steers gave an average creatine content of 1.9771 per cent of the fat-free dry matter, while the exercised steers gave an average of 1.8808 per cent, a difference of 0.0963. The standard deviation from these two averages is 0.1260, and the value of  $t$  is 2.644; for this value of  $t$  and  $n$  is 46, the probability desired is about 0.01. It may, therefore, be concluded that the preponderance of evidence shows that prolonged muscular training has lowered the creatine content of the muscular tissues generally.

The creatine calculations expressed as percentages of creatine nitrogen on the total nitrogen do not reveal any more distinct differences than do the percentages of creatine in the fat-free dry matter.

It is noteworthy that the liver does not show any significant differences in creatine content on any basis of calculation, in contrast with all other comparisons, in which the effect of muscular training was more marked and clear cut with respect to the liver than with respect to any other tissue. This is true not only for the data of experiment 1, but also for the data of experiment 2.

In experiment 2, as in the first experiment, the special sample of round muscles shows the most marked effect of muscular training. The percentages of creatine on the protoplasmic basis show an average difference of 0.036, a standard deviation of 0.0115, and a value of  $t$  of 4.43, indicating a probability of considerably less than 0.01 that a chance combination of factors common to both groups of steers accounts for the average difference observed. This probability is small enough to be neglected. For the same sample, the average percentages of creatine on the fat-free and water-free basis for the two groups differ by 0.14, the standard deviation is 0.0616, the value of  $t$  is 3.21, and the probability is about 0.019 that chance factors only were responsible for the group difference. Again, this is small enough to be neglected. The probability that chance factors accounted for the difference between average percentages of creatine for the heart is about 0.05.

If a single analysis is made of the creatine results for the round, ribs, and shank, the probability that the average percentages on the protoplasmic basis are the result of common factors only is about 0.10, while, for the average percentages on the fat-free dry matter, the probability is 0.02 ( $t=2.52$ ,  $n=22$ ). The latter probability is sufficiently low to neglect. It may be concluded, therefore, that in

this as well as in the other experiment, the data indicate clearly that prolonged muscular training depresses the creatine content of muscle, an effect that is most clearly discernible only when variations in the fat and water content of the muscle have been eliminated by computing the creatine content as a percentage of the fat-free dry matter.

In order to determine the extent to which creatine percentages on the protoplasmic basis in one muscle sample were related to creatine percentages in other muscle samples from the same carcass, correlations were made between deviations of each group of four percentages from its own mean, and deviations of all other groups, making all possible pairings of deviations relating to the same steer. In experiment 1 288 such pairings were made from all the muscle samples except the heart, and the correlation coefficient is  $+0.206$ , perfect correlation being represented by  $+1$ . For the four muscle samples of experiment 2, there were 48 possible pairings, and the coefficient of correlation is  $+0.406$ . Putting its rightful emphasis upon the former coefficient, it may be concluded that only a slight correlation exists between the creatine content of one muscle and that of another in the same carcass.

#### COLLAGEN AND ELASTIN CONTENT OF THE MUSCLES

The results of the determinations of collagen and elastin in the muscle tissue, expressed as collagen and elastin nitrogen in per cent of the total nitrogen, are summarized and averaged in Table 8.

TABLE 8.—*Collagen and elastin contents of the tissue samples from steers on heavy and on light work and from unexercised steers*

EXPERIMENT 1, HEAVY WORK, COLLAGEN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Treatment and steer No.	Heart	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
			Semi- mem- bran- eous	Semi- ten- dino- sus	Biceps femoris	Longis- simus dorsi	Psoas major	Longis- simus dorsi	Obli- quus exter- nus ab- dominis	Triceps brachii
Exercised:										
84.....	11.6	7.5	5.5	5.2	4.6	3.1	4.6	2.9	6.7	5.5
9.....	9.3	13.6	6.3	7.7	7.0	5.9	6.8	4.4	10.2	7.4
62.....	9.5	4.0	6.3	11.8	8.4	5.1	5.3	4.4	8.1	6.6
34.....	13.3	7.8	5.3	14.0	6.9	4.8	10.4	4.6	9.2	10.3
Average.....	10.9	8.2	5.8	9.7	6.7	4.7	6.8	4.1	8.6	7.4
Unexercised:										
23.....	12.8	8.5	5.4	5.2	4.8	3.2	4.2	3.1	6.0	6.3
21.....	11.8	7.5	6.4	8.1	9.1	6.8	6.6	5.6	7.3	8.3
43.....	12.8	4.1	7.1	10.7	9.3	5.8	7.6	5.8	8.9	8.3
36.....	11.9	8.5	5.0	11.4	7.8	4.4	9.1	5.6	8.6	10.1
Average.....	12.3	7.2	6.0	8.8	7.8	5.0	6.9	4.9	7.7	8.2

EXPERIMENT 1, HEAVY WORK, ELASTIN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Treatment and steer No.	Heart	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
			Semi- mem- bran- eous	Semi- ten- dino- sus	Biceps femoris	Longis- simus dorsi	Psoas major	Longis- simus dorsi	Obli- quus exter- nus ab- dominis	Triceps brachii
Exercised:										
84.....	0.26		0.070	1.7	0.23	0.062	0.102	0.056	0.143	0.095
9.....	.32	0.16	.112	2.5	.10	.042	.081	.046	.100	.042
62.....	.30	.11	.082	2.0	.16	.051	.057	.095	.087	.110
34.....	.11	.26	.116	3.3	.10	.080	.108	.049	.200	.079
Average.....	.25	.18	.095	2.4	.15	.059	.087	.062	.132	.082
Unexercised:										
23.....	.36	.12	.112	2.8	.20	.077	.089	.055	.135	.106
21.....	.38	.14	.098	3.0	.14	.064	.063	.075	.090	.047
43.....	.35	.13	.082	2.9	.16	.054	.049	.056	.112	.100
36.....	.32	.09	.084	2.4	.13	.074	.058	.044	.136	.072
Average.....	.35	.12	.094	2.8	.16	.067	.065	.058	.118	.081

TABLE 8.—*Collagen and elastin contents of the tissue samples from steers on heavy and on light work and from unexercised steers—Continued*

## EXPERIMENT 2, LIGHT WORK, COLLAGEN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Treatment and steer No.	Heart	Special sample of round	Round	Ribs	Shank	Loin end	Short loin	Rump	Plate	Chuck	Flank
Exercised:											
80.....	7.5	5.5	12.4	11.2	-----	8.6	11.5	11.2	15.4	15.7	13.6
116.....	8.8	5.2	9.5	12.1	-----	9.7	9.3	9.5	14.3	14.3	14.1
119.....	7.8	5.3	12.8	13.7	24.7	8.4	11.6	11.1	15.6	15.5	12.4
125.....	8.3	4.2	12.0	7.8	23.1	12.0	11.2	12.4	14.4	16.1	12.6
Average.....	8.1	5.0	11.7	11.2	23.9	9.7	10.9	11.0	14.9	15.4	13.2
Unexercised:											
103.....	-----	8.4	10.7	13.8	23.3	11.4	12.2	13.2	12.8	14.8	-----
117.....	11.8	5.1	12.6	13.4	26.4	11.6	11.2	11.0	16.0	15.7	14.9
121.....	8.0	7.9	11.1	8.3	21.1	10.5	11.8	11.6	14.4	16.3	16.4
129.....	7.8	5.3	12.0	13.1	21.6	11.7	10.2	13.4	14.0	16.3	10.6
Average.....	9.2	6.7	11.6	12.2	23.1	11.3	11.4	12.3	14.3	15.8	14.0

## EXPERIMENT 2, LIGHT WORK, ELASTIN NITROGEN AS PERCENTAGE OF TOTAL NITROGEN

Exercised:											
80.....	0.20	0.082	0.48	1.03	-----	0.16	0.43	0.26	2.0	0.49	1.71
116.....	.22	.110	.37	.81	0.08	.12	.22	.18	1.2	.40	.69
119.....	.20	.116	.42	.92	.25	.18	.40	.20	1.6	.40	1.33
125.....	.20	.062	.47	.94	.36	.11	.38	.20	1.4	.49	1.08
Average.....	.20	.092	.44	.92	.23	.14	.36	.21	1.6	.44	1.20
Unexercised:											
103.....	-----	.118	.58	.99	.10	.20	.43	.28	2.1	.57	-----
117.....	.36	.180	.29	.93	.15	.17	.46	.21	1.6	.49	1.34
121.....	.39	.101	.25	.70	.20	.14	.29	.21	1.2	.43	.96
129.....	.19	.097	.71	1.10	.32	.10	.36	.30	2.0	.51	1.09
Average.....	.31	.124	.46	.93	.19	.15	.38	.25	1.7	.50	1.13

It was evident during the making of these determinations that the collagen results were too high, due to the inclusion in the gelatin filtrates of nongelatin material identified by its content of tryptophane. The determinations of experiment 2 were run in no orderly fashion, but those of experiment 1, undertaken the following year, were run in pairs, so that, for each muscle tissue, a sample from an exercised and an unexercised steer were run simultaneously. In each determination, the gelatin filtrate was tested for tryptophane by the Ehrlich colorimetric reagent, and each test was graded roughly according to the intensity of color as faint, medium, and strong. When these tests were assembled and compared at the end of the experiment, it was evident that there was a strong tendency for determinations made simultaneously to give similar tryptophane colorations in the gelatin filtrates. Of 39 such simultaneous determinations on which color gradings were recorded, 37 were given equal gradings and only 2 unequal gradings. It thus appears that, although all collagen determinations are too high, those run simultaneously, having been submitted to more nearly equal conditions of time and temperature, contain similar contaminations of nongelatin nitrogen in the gelatin filtrate and hence are comparable.

The steers in experiment 1 were paired for collagen determinations as follows: Nos. 84 and 23, 9 and 21, 62 and 43, and 34 and 36.

When the determinations are thus paired, and the result for the exercised steer of each pair is subtracted from the result for the unexercised steer, the differences collected in Table 9 are obtained.

TABLE 9.—*Differences in the percentage of collagen nitrogen between the tissues from pairs of steers upon which determinations were made simultaneously, Experiment 1, heavy work*

Pair composed of steers, Nos.	Heart	Special sample of round	Round muscles			Short loin		Ribs	Flank	Chuck
			Semi-membranosus	Semitendinosus	Biceps femoris	Longissimus dorsi	Psoas major	Longissimus dorsi	Obliquus externus abdominis	Triceps brachii
84 and 23.....	1.17	1.05	-0.14	0.04	0.18	0.09	-0.34	0.21	-0.70	0.73
9 and 21.....	2.52	-6.10	.11	.41	2.06	.96	-.25	1.12	-2.82	.87
62 and 43.....	3.32	.13	.80	-1.09	.86	.72	2.29	.94	.56	1.70
34 and 36.....	-1.46	.72	-.28	-2.53	.89	-.40	-1.30	1.02	-.62	-.22

It will be noted that, of the 40 differences, 26 are positive and 14 are negative, indicating that in general the muscles from the exercised steers contained less collagen than the muscles from the unexercised steers. Furthermore, one of the negative differences involves an aberrant result on the special round sample of one of the exercised steers (No. 9), a result that is much higher than any other and may represent a different type of muscle sample than the others, including a different selection of muscles or possibly the inadvertent inclusion of more tendinous tissue.

If all of the differences, with the exception of the difference above noted representing the special round comparison of steers 9 and 21, are averaged, a result of  $+0.29 \pm 0.13$  is obtained. Since this difference is only a little more than twice its probable error, it is not statistically significant, but it is nevertheless suggestive of an effect of muscular training.

Among the comparisons of collagen analyses on the individual muscles, only two, that for the biceps femoris of the round and the longissimus dorsi of the ribs, indicate a significant difference between exercised and unexercised steers or a difference approaching significance. In the case of the biceps femoris, the average difference is +1.00, the standard deviation 0.676, the ratio of the two,  $z$ , is 1.5, and the probability according to Student is only 0.04 that the average is a result of chance. For the longissimus dorsi muscle of the ribs, the average difference is +0.82, the standard deviation 0.36, the ratio  $z$  is 2.3, and the probability is only 0.014. If the differences representing the longissimus dorsi muscle in the rib cut and in the short loin are combined, the mean difference is +0.71, the standard deviation 0.53,  $z$  is 1.33, and the probability that the mean is the result of a random combination of factors common to both groups of steers is only 0.0049. This and the preceding probability are so small as to be negligible, so that it may be concluded that for this muscle at least the evidence is clear that muscular training has decreased the collagen content. In the case of no muscle was there any indication approaching significance that muscular training had increased the percentage of collagen nitrogen.

The elastin analyses of experiment 1 do not indicate any general significant effect of muscular training, except with respect to the heart muscle. Here, in every pair of analyses, the result on the unexercised steer was greater than that on the exercised, the average difference being 0.10 per cent. The standard deviation of the four differences is 0.0634, the ratio  $z$  is 1.66, and the probability that chance produced the average difference is only 0.033.

In experiment 2, involving the lighter intensity of work there are no clear indications of an effect of muscular training, although again there are suggestions that the percentage of collagen nitrogen, and also of elastin nitrogen, have been depressed by work. In 8 of the 11 samples of muscle analyzed, the average percentage of collagen nitrogen was lower for the exercised steers than for the unexercised, and in 9 of the 11 samples the average percentage of elastin nitrogen was lower for the exercised than for the unexercised steers. However, the variations among individual analyses are too great to permit any positive interpretation of these average differences.

An interesting confirmation of these results on the collagen contents of the muscles of exercised and unexercised steers are the results obtained on the tenderness of the cooked ribs obtained from the half carcasses not submitted to chemical study. This work was done by the United States Department of Agriculture. A committee trained in the grading of the various factors contributing to the palatability of cooked meat, including its tenderness, tested all of the 16 rib roasts<sup>6</sup> from the steers of this experiment. In addition, the cooked roasts were subjected to a mechanical test for tenderness employing the method described in Technical Bulletin 217 of the United States Department of Agriculture (2). Through the courtesy of the department the writers have been permitted to refer to these results here in so far as they relate to tenderness. In a series of roasts cooked by a carefully standardized method as these were, it is to be expected that tenderness would vary inversely with the collagen content of the raw meat.

In 1929, when the experiment involved the lesser degree of exercise, there was an indication that the meat from the exercised cattle was more tender than that from the unexercised cattle. This conclusion is based upon both the average committee gradings and the average resistances of the meat to the mechanical device. However, variation among individual roasts, together with the small number involved in each group, detracts from the significance of this indication.

In the 1930 experiment, when there was much more exercise involved and the eight animals were paired, there was a greater difference in tenderness between the exercised and unexercised cattle as shown by the average committee gradings and mechanical tests on the cooked roasts. With three of the four pairs the judges found the meat from the exercised cattle more tender than that from the unexercised cattle. With the other pair the difference was too small to be regarded as of any significance. For that pair of roasts the mechanical test showed, in fact, that exercise had made the meat less tender. With this exception the mechanical test supported the gradings by the judges.

In addition to the roast, a round steak was cooked from each of the 16 cattle in the two years' work. In 1929 the steaks were from the

<sup>6</sup> These rib roasts were taken from the half carcasses not submitted to chemical analysis.

heel of the round and were somewhat lacking in uniformity, due to variations in cutting. For this and other reasons it was impossible to cook (broil) the steaks by a method as well standardized as that employed in roasting the rib cuts. Therefore the value of the data may be questioned but they suggest results in accord with those obtained from the rib cuts from the same experiment.

The round steaks in 1930 were uniformly cut and were taken from a more desirable part of the round than were those of 1929. Again the method of broiling was not standardized, but it is believed that there was greater uniformity in the cooking of the eight steaks than in 1929. Considering both committee gradings and mechanical tests of tenderness the data suggest that the steaks from the exercised cattle were slightly more tender than those from the unexercised cattle.

Any general statement of results must take into account the small total number of animals involved, the variability observed, and the lack of precision in the methods available for measuring tenderness. Making allowance for them, the data indicate that exercise was a factor contributing to tenderness, but the results can not be looked upon as altogether conclusive. When exercise was light, the comparative figures suggested that exercise increased tenderness; when exercise was heavy, the comparative figures showed a slightly greater association of tenderness with exercise.

#### ASH CONTENT OF THE TISSUES

The tissue samples obtained in experiment 1 were analyzed for total ash, but since the results were very uniform and disclosed no effect of muscular training they will not be given in detail. Expressed on the protoplasmic basis, the ash content of the heart samples averaged 1.075 per cent, that of the livers, 1.435 per cent, and that of the 72 muscle samples 1.145 per cent. The coefficient of variation of the latter group of results was only 4.45.

#### SUMMARY AND CONCLUSIONS

The effect of muscular activity on the chemical composition of the tissues of Hereford steer calves has been investigated in experiments covering 2 years. Each experiment involved 8 calves, 4 closely confined and 4 exercised daily on a treadmill. The exercise was continued for 122 days in one year and for 131 days in the other. In one experiment the muscular work imposed was much more intense than in the other, the working period averaging about 3 hours daily as compared with 1 hour daily. All steers were slaughtered at weights of 750 to 850 pounds, and samples of blood, heart, liver, and muscles were taken for analysis. In one experiment the total lean from each of the nine wholesale cuts from one-half of the carcass, separated from all visible fat by knife, constituted the muscle samples. In the other experiment, eight selected muscles were analyzed separately, and in both experiments a special sample of the inner and upper round were taken from the fresh carcasses for immediate study. The results obtained warrant the following statements with reference to the effect of long-continued muscular activity on the chemical characteristics of beef muscle and of other tissues:

No effect on the water, iron, hemoglobin, or red-cell content of the blood was produced.

No effect on the iron and hemoglobin content of the tissues was noted except the iron content of the bone marrow. In the one experiment (light work) in which this tissue was examined, muscular work clearly and markedly reduced the iron content.

While the results of the fat determinations suggested that muscular work tends to lower the content of ether-soluble material in all tissues, only the liver showed a clear and decisive effect, evident in both experiments. The results for the heart muscle were more nearly decisive than those for any other muscle sample.

In the experiment involving the heavier degree of work the evidence is clear that work lowers the content of water in liver, heart, and skeletal muscle, the effect on the liver being the most pronounced. The lighter work markedly lowered the water content of the liver, but its effect on the water contained in the muscles was less evident than in the other experiment. These findings were revealed by calculating the water content to the fat-free, so-called protoplasmic, basis.

No evidence was obtained that the condition of the water in muscle, that is, whether bound or free as measured by the heat-of-fusion method, was at all affected by muscular work.

The heavier degree of work distinctly lowered the nitrogen content of the fat-free dry substance in the liver and exerted a general though less distinct depression of this percentage in the muscles. The effect of the lighter degree of work was only evident in the liver. It appears probable that this general reduction in the nitrogen content of the dry substance of the tissues produced by the heavier degree of work is the result of three changes: (1) A decrease in nitrogenous extractions; (2) an increase in glycogen; and (3) an increase in lipoids not extractable by ether.

In both experiments the evidence indicates clearly that prolonged muscular training depresses the creatine content of muscle, an effect that is most clearly discernible only when variations in the fat and water content have been eliminated by computing the creatine content as a percentage of the fat-free dry substance. The muscle sample taken from the carcass immediately after slaughter and analyzed immediately showed this effect of work more clearly than did the muscle samples taken from the chilled carcasses.

Muscular work tends to lower the collagen content of muscle, expressed as a percentage of collagen nitrogen on the total nitrogen. Possibly the muscular work induced a hypertrophy of muscle tissue with no corresponding increase in the connective tissue elements.

The ash content of the tissues was not appreciably affected by the kind and degree of muscular work imposed in these experiments.

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# THE DEVELOPMENT AND FUNCTIONAL ACTIVITIES OF THE ALBINO RAT AS AFFECTED BY DIETS DEFICIENT IN IRON<sup>1</sup>

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## INTRODUCTION

This study seeks to throw light on the general subject of the functions of iron in nutrition. The experimental animal was albino rat, and the iron was supplied in natural foodstuffs. An iron-poor diet, the source of the protein and the iron of which is dried skim-milk powder, is compared with an iron-rich diet containing meat, as affecting the growth, the fasting metabolism, the blood composition, and the reproduction of the rat; and also as affecting the histology of the liver, the kidney, the spleen, the skeleton, and the muscle of this species.

## REVIEW OF LITERATURE

Milk is a food having a characteristically low iron content. This was reported by Bunge (10).<sup>3</sup> Abderhalden (1) first showed that an animal fed an exclusive milk diet developed anemia. Hart, Steenbock, and associates (22; 23; 24; 51; 11, p. 73-75), Beard and his associates (5, 6, 7, 8, 43, 44), Titus and Hughes (50), and Krauss (26, 27, 28, 29) are among the recent investigators who have produced nutritional anemia by the exclusive feeding of liquid whole milk to rats. Whipple and Robschheit-Robbins (52) have demonstrated the ineffectiveness of milk to cause blood regeneration in dogs made anemic by bleeding, and Miller, Forbes, and Smythe (40), and Miller and Forbes (39) have shown that the iron content of the bodies of rats becomes subnormal as a result of feeding on diets in which the iron and protein is supplied solely by dried milk.

In spite of the fact rats fed milk are anemic, with less than normal amounts of iron in their bodies, the growth of rats on milk diets after weaning is not much less than the normal. Also during the suckling period the mother's milk supports rapid growth but does not induce much iron storage.

According to Abderhalden (2) and Williams and Ets (53), the percentage of hemoglobin in rat's blood diminishes during the suckling period, when the sole food is the mother's milk. Smythe and Miller (49) found a decrease in the percentage of total iron in the bodies of suckling rats, though the absolute amount of iron increased somewhat during the same period. After weaning, the percentage and absolute amounts of iron in the rats' bodies both increased rapidly. The iron content of females was depleted during pregnancy but

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<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 959.

returned to normal after the young were weaned. These observations of Smythe and Miller on suckling rats have been confirmed by Lintzel and Radeff (30).

It is recognized, on the strength of evidence to be cited, that exclusive milk diets do not adequately support reproduction, and there is some evidence that the low iron content of milk is, at least in part, responsible for its nutritive deficiency in this respect.

Waddell, Steenbock, and Hart (11) found that when milk was fed to rats as the sole food, fortified only with copper and iron, living young were produced to the third generation, although both growth and reproduction were diminished. When small amounts of manganese, or iodine, or both, were added to the diet reproduction improved greatly.

Krauss (29) did not obtain any reproduction when rats were fed an exclusive whole milk diet. The addition of copper; iron and copper; iron, copper, and yeast; iron, copper, and wheat-germ oil; yeast alone; or cane molasses permitted reproduction, but this was in no case normal. Lactation was poor.

Daniels and Hutton (14) overcame the reproductive difficulty accompanying exclusive milk feeding by using a complex mineral supplement containing aluminum, potassium, sodium, fluorine, silicon, manganese, iron, and iodine.

Anderegg (3) obtained reproduction and lactation while feeding whole milk powder with supplements of iron citrate, 0.2 per cent, and agar-agar, 4.8 per cent. Later Anderegg and Nelson (4) made a study to determine the supplements necessary to make whole and skim milk powders adequate to support reproduction. Whole-milk powder properly supplemented with iron salts and carbohydrate, or with iron salts alone, supported good growth, reproduction, and rearing of young, the amount of iron salts added having a pronounced effect upon the reproduction. Skim-milk powder required an additional supplement of either yeast or wheat embryo before successful reproduction could be obtained.

Clayton (12) credits part of the success of Anderegg to the ash content of the agar-agar.

Mattill and Conklin (35) observed that when weanling rats were placed on a liquid milk diet no reproduction occurred, even though good growth resulted for 50 or 100 days; neither did the use of dried whole milk or dried skim milk, to which butter was added, favorably influence reproduction. Examination of the testes showed that motile spermatozoa were present. The ovaries were 50 per cent below normal weight. The authors stated that possibly milk is lacking both quantitatively and qualitatively in substances necessary for successful adolescent growth and for reproduction, especially in the female. Later Mattill and Stone (36) found reproduction unsuccessful in both male and female rats receiving rations in which all of the protein and known vitamins were supplied by varying proportions of dried whole milk.

Still later Mattill, Carman, and Clayton (34) found that a milk diet high in fat did not support reproduction in rats, but that the addition of 5 to 10 per cent of wheat embryo restored reproductive powers. When a milk diet low in fat was used, marked reproductive failure did not appear.

Sherman and Muhlfeld (48) demonstrated that as the proportion of whole-milk powder was increased in a diet already adequate for growth, reproduction, and lactation, there resulted increased fertility among females, an increase in the number of offspring, an increase in the size attained by the young during suckling, and a longer period of reproduction. These findings, therefore, show that while milk used as the sole food is nutritively adequate to support growth, its deficiency in iron, and in other factors, results in deficient nutrition of the blood, and possibly also in subnormal reproduction.

Meat as compared with milk has a relatively high iron content, and its usefulness in the nutrition of the blood and in curing secondary anemias is well established. The literature concerning iron in this relation has been reviewed by Hall (20), Meyer (38), Robschey-Robbins (47), and Krauss (29).

Miller and Forbes (39) have reviewed the literature pertaining to the value of meat protein for growth, and for reproduction.

The present investigation was concerned with the relative usefulness of meat and milk as sources of iron for growth, nutrition of the blood, and reproduction, and as affecting heat production and the histology of the tissues.

#### EXPERIMENTAL PROCEDURE

An outline of the schedule of experimentation is presented in Table 1. The experimental subjects were albino rats from the colony of the Institute of Animal Nutrition of the Pennsylvania State College.

Experimental feeding began immediately after weaning, at which time the rats were 24 to 28 days old and weighed from 35 to 45 g. These rats were the offspring of females that had been fed the stock diet, the composition of which is given in the following tabulation:

Stock ration:	Parts
Yellow corn.....	25
Wheat red-dog flour.....	22
Oat flour.....	15
Linseed meal.....	15
Blood flour.....	10
Ground malted barley.....	10
Sodium chloride.....	1
Precipitated calcium carbonate.....	1
Precipitated bone.....	1
Cod-liver oil.....	5
Wheat-germ middlings.....	5
Total.....	110

This ration contained 22 per cent of crude protein and 0.027 per cent iron. Part of the stock from which the rats were derived had been fed this diet during the last 14 generations. In addition, wheat-germ oil was given to the females once daily, with a medicine dropper, during the latter half of the growth period and throughout the periods of breeding and pregnancy.

The experimental rats were fed individually in galvanized-wire cages provided with wire false bottoms to prevent access to excreta. Each rat had access to fresh tap water at all times.

The food intake was controlled in all animals, except the females during gestation and lactation, when they were allowed as much food as they would consume; otherwise all rats were fed weighed daily portions of feed, seven such portions being weighed at one time and stored in tin boxes until used.

TABLE 1.—Schedule of experimentation

Generation of animals, experimental series, and date	Diet No.	Number and sex of rats	Duration of experi- mental feeding	Protein feeds	Experimental treatment
First generation:			Weeks		
Series 1— Started Oct. 19, 1928.....	23	{ 4 females... 4 males... }	15	Skim-milk powder.	All animals used for the determination of heat production and blood constituents; 4 females used for reproduction; 4 males used for histological investigation.
	24	{ 4 females... 4 males... }	15	{ Skim-milk powder and beef muscle. }	Do.
Series 2— Started Mar. 23, 1929.....	23	5 females...	15	Skim-milk powder.	Used for testing reproduction.
	24	---do-----	15	Skim-milk powder and beef muscle.	Do.
Series 3— Started Jan., 1930.	23	15 females...	15	Skim-milk powder.	Used for testing reproduction
	24	16 females...	15	Skim-milk powder and beef muscle.	and blood constituents. Do.
Second generation: Females from series 1, 2, and 3.....	23	11 females...	15	Skim-milk powder.	Do.
	24	18 females...	15	Skim-milk powder and beef muscle.	Do.

The rats received the vitamin supplements separately, pellets of Harris Yeast Vitamin being given daily in the feed cups, and cod-liver oil daily with a medicine dropper.

For the growth experiments the rate of food allowance was determined by the food consumption of the group of rats eating the smaller amount, the quantity of food eaten by this lot, in relation to its live weight, constituting the bases for the allowance of feed to the other lot. The quantities of food thus assigned are presented in Table 2, the make-up of the rations and the chemical composition of the diets in Table 3. The experience gained from this method of feeding, in the experiments of series 1 of the first generation, made possible the more successful regulation of the food in the succeeding experiments with both generations. The rats were weighed at weekly intervals, and the quantity of food given was computed on the basis of these weekly weights.

TABLE 2.—Numerical basis of control of food intake in grams eaten per week per gram of body weight

Week No.	First generation						Second generation	
	Series 1		Series 2		Series 3		From first-generation series 1, 2, and 3	
	Diet No. 23	Diet No. 24	Diet No. 23	Diet No. 24	Diet No. 23	Diet No. 24	Diet No. 23	Diet No. 24
1.....	0.62	0.65	0.84	0.78	0.78	0.82	0.76	0.92
2.....	.69	.70	.64	.63	.66	.64	.61	.64
3.....	.70	.59	.55	.56	.57	.59	.57	.57
4.....	.50	.54	.53	.49	.49	.51	.53	.51
5.....	.47	.46	.48	.48	.49	.48	.49	.47
6.....	.43	.41	.42	.42	.43	.42	.41	.44
7.....	.39	.38	.39	.38	.39	.39	.38	.39
8.....	.39	.34	.35	.38	.35	.37	.35	.36
9.....	.34	.31	.33	.34	.32	.32	.31	.32
10.....	.32	.30	.31	.31	.31	.31	.29	.31
11.....	.30	.30	.30	.30	.30	.30	.29	.30
12.....	.28	.29	.29	.28	.28	.29	.28	.29
13.....	.29	.27	.27	.27	.27	.27	.27	.27
14.....	.26	.28	.25	.25	.25	.25	.25	.27
15.....	.25	.24	.25	.25	.25	.25	.25	.25

TABLE 3.—Composition of diets <sup>a</sup>

Components and constituents of diets	Diet No. 23 <sup>b</sup>	Diet No. 24 <sup>c</sup>
Components:	<i>Per cent</i>	<i>Per cent</i>
Skim-milk powder.....	44.37	44.37
Dried beef muscle.....		20.00
Lactose.....	10.00	10.00
Starch.....	31.95	21.03
Crisco.....	13.18	4.10
Sodium chloride.....	.50	.50
Fat.....	13.60	10.00
Constituents:		
Crude protein.....	15.00	27.00
Iron.....	.001	.002

<sup>a</sup> Cod-liver oil and Harris Yeast Vitamin were given daily, apart from the ration.<sup>b</sup> Energy value, 494.7 calories per kilogram.<sup>c</sup> Energy value, 494.5 calories per kilogram.

When this method of food control is used, the apparent palatability of the diet, which serves to determine the rate of feeding, may be fundamental, in the sense of being truly characteristic, or may be a secondary result of nutritive deficiency and consequent low state of nutrition. In this investigation the quantity of food consumed in proportion to the body weight became smaller each week until the maximum body weight was attained.

There is room for question, however, as to the equitableness of this method of feed apportionment. Thus, since the maintenance requirement of the smaller animal is greater in proportion to body weight than is that of the larger one, a smaller quantity of food in proportion to body weight is left for gain. In this respect, the larger animal is at a comparative advantage; but in another the advantage may rest with the smaller animal, which has thriven less well, since it may have less desire for food than has the larger animal, which has made the better growth. The larger animal, therefore, perhaps suffers from an unsatisfied appetite whereas the smaller animal does not. Since the comparative reaction of the two animals may differ with the nature of the ration it is impossible to say, in general terms, which animal enjoys the net advantage.

At the termination of the 15 weeks feeding for growth, the rats were used for other phases of the study.

The beef muscle fed was purchased in the local market. The lean portion was freed from the bone and fat and was ground twice in a food chopper made of nearly iron-free phosphor-bronze. The finely ground fresh muscle was placed in a glass drying dish and dried in a Freas oven at about 85° C. The dried material was then further ground in the phosphor-bronze chopper.

In planning the feeding treatments the iron-poor diet (No. 23) was first adopted, dried skim milk being the sole source of protein and iron; then the iron-rich diet (No. 24) was devised by substituting dried beef muscle for an equi-caloric portion of the nonnitrogenous components of the iron-poor diet. The two diets, therefore, were balanced as to energy value, but not as to protein content. The diets were not balanced as to protein because the protein of the iron-poor diet (dried skim milk) was considered to be fully adequate, both as to quantity and composition, and it would be theoretically impossible for the added dried beef muscle either to augment or to diminish the protein value of the diet. Also inasmuch as vitamin and mineral

nutrient requirements (other than iron) were fully satisfied by both diets, the basis was established for the demonstration of the additional nutritive value of diet 24, as compared with diet 23, presumably on account of its higher iron content, but possibly on account of other constituents of the meat.

The iron content of the diets was determined by the modified thiocyanate-acetone method, which is discussed in detail by Miller, Forbes, and Smythe (40).

### GROWTH

The data representing food intake and body gain are given in Table 4, while curves representing average growth, and histograms of total weekly food consumption are given in Figure 1.

Approximately normal growth was obtained from both diets throughout the three series, but the rats fed the high-iron diet (No. 24) made a slightly greater increase in body weight in each case.

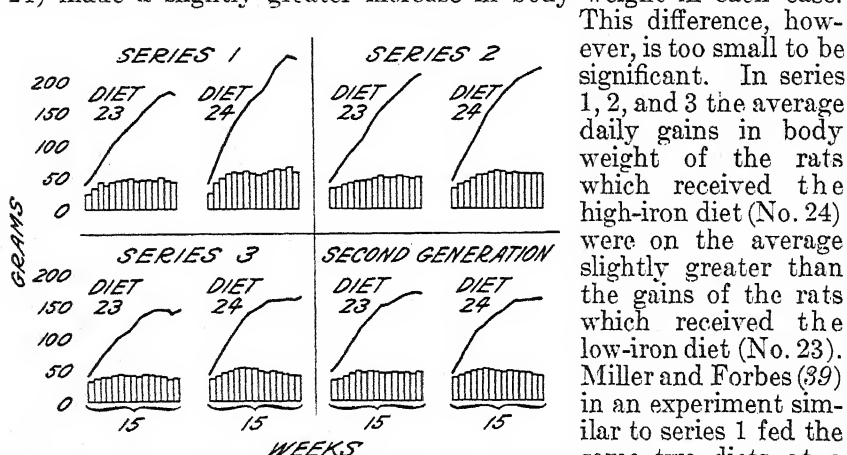


FIGURE 1.—Average growth (curves) and average food intake (bars) of rats on iron-deficient (No. 23) and high-iron (No. 24) diets

slightly greater average daily gain in body weight with the group fed the low-iron diet.

The food consumption per gram of body weight was practically the same for the rats of both groups in all experiments; and the iron intake of the rats fed the high-iron diet was more than twice that of those fed the low-iron diet.

The findings of all three series are similar in respect to growth. The composite curves in Figure 1 show that the average rate of gain was lower for the rats of series 3 (first generation) and for the rats of the second generation than the average rates of gain for the rats of series 1 and 2 (first generation), which may possibly have been the result of the very warm weather which prevailed during the feeding of the groups first mentioned. This suggestion is supported by the experience of Hanson and Hays (21) who found, in a study of the growth of rats during different seasons of the year, that females born during the fall have the greatest mean body weight at all ages and that those born during the summer have the smallest body weight.



TABLE 4.—*Foods and iron consumed and gains in body weight*

Generation and number of animals and experimental series	Sex	Diet No.	Source of protein and iron	Average daily gain in body weight	Average daily food intake	Average daily iron intake	Average weekly food intake per gram of body weight
First generation:							
Series 1—				Grams	Grams	Milli-gram	Gram
4.....	Male.....	23	Skim-milk powder.....	$1.48 \pm 0.09$	6.49	0.07	0.42
4.....	Female.....	23	Skim-milk powder.....	$1.17 \pm .03$	6.04	.06	.42
4.....	Male.....	24	Skim-milk powder and beef	$2.22 \pm .07$	8.75	.18	.41
4.....	Female.....	24	muscle.....	$1.43 \pm .07$	6.82	.14	.40
Series 2—							
5.....	do.....	23	Skim-milk powder.....	$1.57 \pm .06$	6.46	.07	.41
5.....	do.....	24	Skim-milk powder and beef	$1.68 \pm .07$	7.26	.14	.41
Series 3—							
15.....	do.....	23	Skim-milk powder.....	$.98 \pm .04$	5.53	.06	.41
16.....	do.....	24	Skim-milk powder and beef	$1.15 \pm .03$	6.49	.13	.42
Second generation—							
11.....	do.....	23	Skim-milk powder.....	$1.20 \pm .04$	5.91	.06	.40
17.....	do.....	24	Skim-milk powder and beef	$1.13 \pm .03$	6.32	.13	.42
			muscle.....				

The food consumption and the average daily gains in body weight of the second-generation females are presented in Table 4 and in Figure 1. Since the number of individuals per treatment in each series of comparison was diminished in the tests of the second generation, as compared with the first, especially among the individuals fed the low-iron diet, the individuals of the three series on each diet were combined to determine the average food consumption and gain in body weight.

No evidence is found in the growth data to indicate that the low-iron diet adversely influenced the gain in body weight during two generations of feeding.

#### HEAT PRODUCTION

One phase of the investigation was to determine the effect of previous feeding with the low-iron and high-iron diets on the fasting heat production.

Meyer and DuBois (37) found an increased demand for oxygen in severe anemia, but no conclusive evidence was found of a definite level of basal metabolism characteristic of anemia.

Pettenkofer and Voit (46) made a study of a severe case of anemia in man, in which they found the metabolism to be exactly the same as for a normal resting man. This investigation, however, was reviewed by Magnus-Levy (33), who concluded that in this case metabolism was increased by anemia. He came to the same conclusion in a study of a severe case of anemia, conducted by himself.

In the present study each rat of the first series of tests was subjected to a measurement of the heat production by means of a modified Haldane apparatus (19).

In the conduct of a respiration experiment a rat was fasted for approximately 24 hours and was then kept for a period of 5 to 6 hours

in the respiration apparatus, during which time the carbon-dioxide production and the oxygen consumption were determined. The heat production as computed from these data, however, could not be considered accurately to represent the basal metabolism of the animal, since the activity of the subject was not controlled. In every experiment the animal spent the greater portion of the experimental period at complete rest, and in no instance was excessive activity observed.

The critical temperature of the rat, on a basal plane of nutrition, is about 28° C., according to Goto (18) and Benedict and MacLeod (9). The present respiration determinations were made during December, 1929, in a room with a fairly constant temperature, varying, however, between 26° and 30°. The rats, when not in the respiration chamber, were kept in the stock-colony room, which was maintained at a temperature of about 27° except during seasons when the normal temperature was above 27°.

The heat production was computed by the modified Zuntz and Schumburg method, as given by Lusk (31), using the total respiratory quotient, i. e., neglecting the katabolism of protein.

The details of the determinations of the heat production, together with information concerning sex, age, length of fast, body weight, and food consumption, are given in Table 5. The body surface areas of the rats were calculated from their body weights by means of the Meeh formula ( $S = KW^{.73}$ ), using the constant 7.47, recently derived by Diack (15). The age and sex of the subjects and the length of the periods of experimental feeding were approximately the same for the two groups. The heat production was determined at the eighth to the eleventh week of experimental feeding.

The average of the respiratory quotients, determined between 16 and 50 hours after the last feeding, was 0.75 (ranging between 0.72 and 0.77) for the low-iron diet (No. 23), and also 0.75 (ranging between 0.72 and 0.80) for the high-iron diet (No. 24).

The figures for the heat production of the rats in this study were calculated on two bases: (1) In relation to the two-thirds power of 100 g of body weight per 24 hours; and (2) per square meter of body surface per 24 hours. On the low-iron diet the average daily heat production of the rats was 15.56 calories per 100 g of body weight and 967 calories per square meter of body surface. For the high-iron diet the average daily heat production of the rats was 15.34 calories per 100 g of body weight, and 953 calories per square meter of body surface.

Benedict and MacLeod (9), using the constant 9.1 in the Meeh formula, found the average so-called basal metabolism of the albino rat, at 28° C. and above, to be about 775 calories per square meter of body surface per 24 hours, when no differentiation is made for sex. For females alone, the figure representing the basal metabolism at 28° and above is about 720 calories, and for males 800 calories.

Mitchell and Carman (41), using rats ranging in weight from 84 to 251 g, reported a fasting metabolism of 600 calories per square meter of body surface per 24 hours for males, and 571 calories for females (at complete rest), having used in the computation of the body surface the constant 11.36, in the Meeh formula.

If 7.47 instead of 9.1 is used as the constant in the surface-area formula, the values of Benedict and MacLeod are 944, instead of 775 calories, where sex is not considered, and 877 calories for females,

and 975 calories for males. The figures of Mitchell and Carman, when recomputed in the same way, are 912 and 868 calories for males and females, respectively.

The average values obtained in this investigation, for the fasting heat production per square meter of body surface per 24 hours are in close agreement with values reported by Benedict and MacLeod (9) and are higher than those obtained by Mitchell and Carman (41). A slightly higher value should be expected in this investigation since the data were taken from rats during periods of five or six hours, with no control over activity, while the above-mentioned investigators obtained values from rats during short periods of complete rest.

The high-iron and low-iron diets, however, did not differ in their effect on subsequent fasting heat production, and the results as given in Table 5 are all in good agreement with those representing normal individuals as reported by other investigators.

TABLE 5.—Data relating to the determination of the heat production of rats on low- and high-iron diets

DIET 23, LOW IRON

Rat No.	Sex	Age	Length of fast	Body weight	Respiration period	Respiratory quotient	Oxygen consumed per day	Heat produced per day		
								Total	Per 100 g body weight <sup>a</sup>	Per square meter of body surface
		Days	Hours	Grams	Minutes		Liters	Calories	Calories	Calories
508.....	Female.....	97	20	135	240	0.75	3.87	18.34	15.01	933
509.....	Male.....	86	20	134	973	.75	4.40	20.85	17.15	1,066
510.....	do.....	89	29	125	994	.74	3.66	17.30	14.91	927
511.....	Female.....	95	24	132	300	.77	3.41	16.25	13.50	839
512.....	Male.....	97	24	171	300	.74	4.77	22.55	15.76	979
513.....	Female.....	98	19	149	353	.72	4.81	22.62	17.34	1,078
514.....	Male.....	80	16	141	300	.76	3.91	18.58	14.78	918
515.....	Female.....	91	24	136	300	.77	4.13	19.68	15.04	997
Average.....		91				.75			15.56	967

DIET 24, HIGH IRON

		Days	Hours	Grams	Minutes		Liters	Calories	Calories	Calories
500.....	Female.....	85	25	141	360	0.80	3.37	16.18	12.86	799
500.....	do.....	86	50	135	300	.78	3.61	17.24	14.11	877
501.....	Male.....	88	22	192	280	.72	5.52	25.96	16.80	1,044
502.....	Female.....	96	25	188	285	.73	5.23	24.65	16.18	1,005
503.....	do.....	93	27	151	300	.75	4.00	18.96	14.41	895
504.....	Male.....	97	20	205	290	.78	5.06	24.17	14.97	930
505.....	do.....	97	22	237	300	.74	5.95	28.13	15.82	982
506.....	do.....	95	27	203	300	.74	5.47	25.86	16.13	1,002
507.....	Female.....	85	22	147	300	.73	4.59	21.64	16.74	1,040
Average.....		91				.75			15.34	953

<sup>a</sup> Computed to correspond to uniform weight, from the total heat production in relation to the two-thirds powers of the body weight.

### BLOOD CONSTITUENTS

The quantity of hemoglobin in the blood was determined, and red and white blood cell counts were made on the animals of series 1 and 3 of the first generation, and also on the rats of the second generation.

Waddell (11) dispensed with the erythrocyte count on the ground that it added little information to that obtained from hemoglobin

determinations and involved considerable extra labor. However, Beard and his associates (7, 43) emphasized the importance of making erythrocyte counts in addition to hemoglobin estimations, inasmuch as erythropoietic stimulants seem to act first on red blood-cell formation.

Although Jencks (25), Waddell (51), Mitchell (42), and McCay (32) successfully obtained blood from the tail of a rat, the writers found it easier to obtain it from a vein (the saphena parva) of the thigh. More blood is obtainable from this vein in a given time than from the tail, and there is less disturbance of the animal.

The procedure used in drawing the blood sample was as follows: The hair is clipped close to the skin of the outside of the thigh just above the knee joint. The place to be punctured is scraped carefully with a sharp knife and then washed and dried. The scraping and

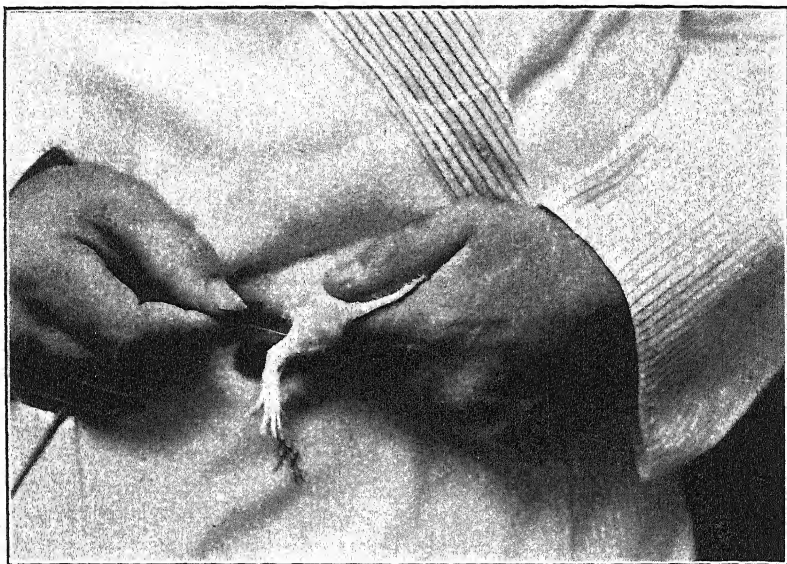


FIGURE 2.—Method of holding rat and securing blood sample from the vena saphena parva

washing are done to remove from the skin substances which hasten the clotting of the blood. Pressure is exerted with a thumb and forefinger, about the hip joint, to increase the blood in the vena saphena parva. This vein is then punctured with a blood lancet, just above the knee joint. It is possible to obtain with one puncture enough blood for the hemoglobin determination and the erythrocyte and leucocyte counts. The method is illustrated in Figure 2.

The samples for determination of blood constituents were taken during the week succeeding the termination of the growth-feeding period. Hemoglobin estimations were made by the acid-hematin method (13), color comparisons being made with a calibrated Newcomer disk (45). A standard counting chamber with the improved Neubauer ruling was used for the erythrocyte and leucocyte counts. The erythrocytes were diluted for counting (1:200) in Hayem's solution (composed of 200 cc of distilled water, 1 g of sodium chloride,

5 g of sodium sulphate, 0.5 g mercuric chloride). The leucocytes were diluted for counting (1:20) in a solution of 1 per cent glacial acetic acid tinged with gentian violet.

The rats on both diets were normal with respect to blood constituents as shown in Table 6.

TABLE 6.—*Blood constituents of rats on low-(No. 23) and high-(No. 24) iron diets*

Diet No.	Generation	Hemoglobin		Erythrocytes		Leucocytes	
		Rats	Average per 100 cc of blood	Rats	Average per cubic millimeter of blood	Rats	Average per cubic millimeter of blood
		Number	Grams	Number	Number	Number	Number
24.....	First.....	24	17.44±0.25	24	8,175,000±111,000	21	7,500±140
23.....	do.....	24	15.37±.13	23	7,772,000±148,000	21	7,560±122
24.....	Second.....	13	16.89±.32	10	9,787,000±205,000	8	7,420±216
23.....	do.....	10	15.95±.28	5	7,874,000±156,000	4	7,950±506

In the first generation the average hemoglobin content of the blood of the rats on the high-iron diet was 17.44 g per 100 cc of blood, as compared to 15.37 g for the rats on the low-iron diet. The difference,  $2.07 \pm 0.28$  g, is statistically significant. In the second generation, the hemoglobin content of the blood of the rats on the two diets was not significantly different from that of the rats of the first generation, and although it was slightly higher for the rats on the high-iron diet as compared to that for the rats on the low-iron diet, the difference was not significant. All the hemoglobin values may be considered normal.

The average number of erythrocytes per cubic millimeter of blood for the rats in the first generation on the high-iron diet was 8,175,000 and for those on the low-iron diet 7,772,000; for the second generation 9,787,000 for those on the high-iron diet and 7,874,000 for those on the low-iron diet. The number of erythrocytes is significantly higher for the rats fed the high-iron diet in the second generation as compared with first generation rats on the same diet, and with first and second generation rats on the low-iron diet. The results with respect to the number of leucocytes in the blood show no significant differences in any of the treatments.

Miller and Forbes (39), feeding the same diets used in this investigation, found that rats fed the high-iron diet (No. 24) stored more iron in the body than did those given the low-iron diet (No. 23), but this difference in total iron stored was much more pronounced than were the differences in hemoglobin and in blood cells as observed in the present study. This fact is significant in relation to the observation of McCay (32, p. 29).

A decrease in the rate of recovery during a long anemic period shows that the usual criteria of blood regeneration may be false. When the cell count and hemoglobin of an anemic animal have returned to normal it is said to be recovered. The recovery is probably only apparent in many cases since the reserves may remain very low.

## REPRODUCTION

After the termination of the 15-week growth experiment, the female rats from series 1, 2, and 3, the treatment of which was indicated in Table 1, became the subjects of a study of the effects of iron deficiency on reproduction. The individuals of each category as to iron content of ration, and generation of young produced, were considered as a separate group in this breeding experiment, there being four such groups. A summary statement of the results of the four groups is given in Table 7.

TABLE 7.—*Summary of results relating to reproduction and lactation of rats on low (No. 23) and high (No. 24) iron diets*

Generation and diet No.	Fe-male experimental subjects	Fe-males that did not reproduce	Total young born	Young born dead	Young that died during suckling period	Young weaned		Average weight of young at birth	Average young per litter at birth	Average weight of young at weaning	Average young per litter at weaning
						Number	Per cent				
First generation:	Number	Number	Number	Number	Number	Number		Grams	Number	Grams	Number
23-----	25	8	117	8	33	76	65.0	4.9	6.9	42.1	5.8
24-----	26	6	168	0	22	142	84.5	5.0	8.4	40.5	7.5
Second generation:											
23-----	11	1	69	15	30	24	34.8	<sup>a</sup> 4.6	<sup>a</sup> 6.9	36.2	6.0
24-----	18	2	133	3	21	105	79.0	4.7	8.3	44.3	6.6

<sup>a</sup> Includes 6 atrophic, partly developed fetuses.

<sup>b</sup> Does not include 6 partly developed fetuses.

The females were mated as soon as possible after the termination of the growth experiment, and each was kept in an individual cage in which she was fed until her young were born and then weaned, 25 females being given the low-iron and 26 the high-iron diet. A daily supplement of wheat-germ oil was fed with both diets to supply vitamin E during the time of the study of reproduction.

In the study of first-generation reproduction, 117 young were produced by 17 females receiving the low-iron diet (No. 23), and 168 young by 20 females receiving the high-iron diet (No. 24). Six females on the high-iron diet and 8 females on the low-iron diet did not produce any young, 1 female in each group having died while pregnant, and the others having failed to become pregnant during at least seven weeks to males previously proved to be fertile. The latter females were removed from the experimental groups and discarded.

The young produced on both diets weighed about the same at birth; but at the time of weaning the average weight of the young whose mothers were fed the low-iron diet was 1.6 g more than that of those whose mothers received the high-iron diet, this difference probably depending on the smaller number of young per litter from the females on the low-iron diet. Not only was the average number of young per litter at birth smaller for the low-iron group than for the high-iron group but so also was the percentage of young that survived during the suckling period. Four females on the low-iron diet failed to wean any young from a total of 27 born alive; while only one entire litter was lost during the suckling period by the females on the high-iron diet. Three of the females given the low-iron diet are known to have killed their young and in part to have eaten them. No dead

young were produced by the females on the high-iron diet, while 8 dead young were born to those on the low-iron diet.

Second-generation females from 11 mothers in the low-iron group and from 18 in the high-iron group were available for a further study of reproduction as affected by the two diets. (Table 7.)

Each second-generation female, from the time it was weaned until it had produced young and weaned them, was given the same diet as that which had been fed to its mother.

One female was discarded from the low-iron group because of failure to breed. Two females on the high-iron diet failed to breed, one of them having developed an abnormal growth of the incisor teeth, the cause of which was unknown. This condition appeared similar to that which develops when an excessive amount of fluorine is present in the diet; however, but one animal was affected, the teeth of the other members of the group appearing to be normal.

In the second generation, on both diets, approximately the same average number of young were produced per litter as in the first generation. The average weight of young at birth was approximately the same for both diets.

On the low-iron diet 34.8 per cent of the total number of young were weaned, 15 individuals having been born dead and 30 having died during the suckling period. In the first generation on this diet 65 per cent of all young were weaned. In both generations on the high-iron diet the females weaned a higher percentage of the total number of young than did the females on the low-iron diet.

While there was not much difference in the size of the rats weaned by females on both diets in the first generation, the average weight of those weaned by the females fed the high-iron diet in the second generation was considerably greater than that of the young weaned by the females of the low-iron group. Only 4 litters were weaned of the 10 produced by the latter group, and the size of the rats in 2 of these was considerably less than normal; on the other hand, 16 litters were produced and weaned by females on the high-iron diet in the second generation, and the weanling rats were normal in size.

Thirteen of the 17 females of the first generation which were given the low-iron diet (No. 23) weaned all or part of their young born. Five of 10 females of the second generation given the low-iron diet had a total of 15 young born dead, 1 female giving birth to 6 atrophic fetuses and 1 live but undersized individual. In the first generation, on the same diet, 4 out of 17 females had a total of 8 young born dead. Thirty young from 10 females of the second generation, fed the low-iron diet, died during the suckling period, as compared with 33 young from 17 females of the first generation fed the same diet.

In both generations the rats fed the low-iron diet had rough coats of fur and also seemed to be low in vitality, as indicated by lack of normal muscle tone. The inadequacy of the low-iron diet was also reflected in the appearance and behavior of the rats. In both generations some of these rats killed and ate a part of their young, but this did not occur in the case of the females on the high-iron diet in either generation.

A typical example of the effect of the iron-deficient diet on reproduction and lactation during the second generation is illustrated in Figure 3. The smaller rat was from a second-generation female on the low-iron diet and represents the average in size of the 8 rats of this



litter at 18 days of age. The larger rat was from a second-generation female on the high-iron diet and is the average in size of the rats of a litter of 14 young at 20 days of age.

It is apparent that the high-iron diet supported reproduction and lactation much more effectively than did the low-iron diet, and that marked nutritive deficiency characterized those rats which received the latter diet.

Since Miller and Forbes (39) found the total iron content of the bodies of rats fed the low-iron diet (No. 23) to be low, it is probable that in the present work the females which received this diet were likewise deficient in the iron content of their bodies, and that this deficiency was responsible for the observed lack of capacity for normally successful reproduction.

The unfavorable effects of iron deficiency on reproduction were more prominent in the performance of the second-generation females con-

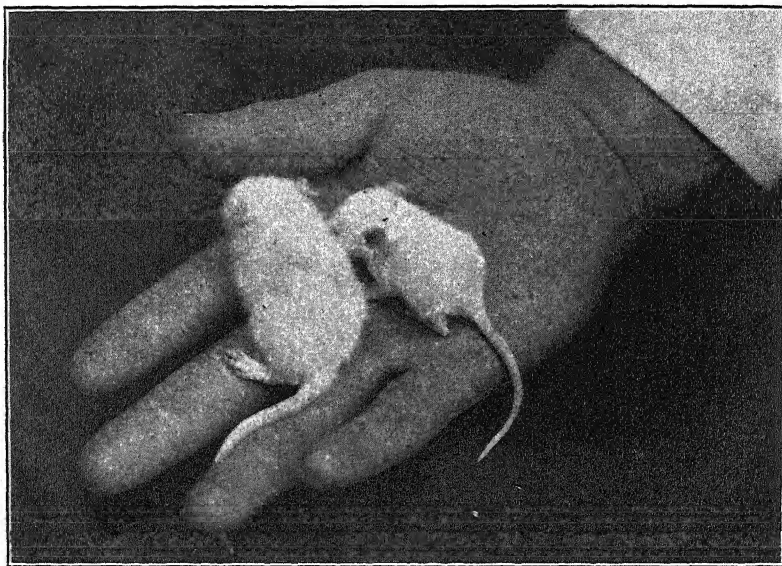


FIGURE 3.—Representative second-generation rats, 20 days old, on high-iron diet, left; 18 days old, on low-iron diet, right

finied to a low-iron diet than in that of the first-generation females—which is understandable in the light of the following facts: The nourishment of the first-generation females on the low-iron diet began at the time of weaning; and the nourishment of the second-generation females on the low-iron diet began in the uterus of the mother at the time of conception. The first-generation females, therefore, were nourished during the periods of pregnancy and lactation by mothers supposedly normal with respect to the iron content of the diet and to the quantity of iron in their bodies, while the second-generation females were nourished by mothers that had been given an iron-deficient diet, beginning at the age of weaning, and were supposedly low in the iron content of the body. The deficiency of iron in the bodies of the second-generation females not only deprived the offspring of iron during embryonal development but also hindered the



normal functioning of the body of the mother during the periods of pregnancy and lactation.

From the performance of the rats while reproducing on the low-iron diet it appears that under this condition they will not sacrifice the iron of their own body tissues to such extent as to cause severe injury for the purpose of nourishing the young in utero. When the iron content of the body of a female is low the iron content of her embryos will be low, the exact quantities received determining whether the young will be absorbed from the uterus, or will be born immature, or will be developed to normal maturity.

#### HISTOLOGICAL STUDIES

A microscopic study was made of sections of the bone marrow, liver, kidney, spleen, and muscle tissue of the male rats of series 1. Since the formation of erythrocytes and hemoglobin involves these tissues, an iron deficiency, if sufficiently pronounced, would be expected to produce abnormalities in their structure.

Doyle, Mathews, and Whiting (16) made a microscopic study of the tissues of pigs which were severely anemic. The most prominent changes were marked fatty degenerative infiltration of the liver, and the presence of hematopoietic centers in the liver, spleen, and bone.

Drabkin and Miller (17) found no evidence of pathological lesions in the intestines of rats which had died from milk anemia.

The samples of tissues for histological study in the present investigation were obtained from the males of series 1 at the termination of the feeding trial. The animals were chloroformed, and the livers, kidneys, spleens, and certain bones and muscles were removed immediately, and placed in Diedrich's solution (composed of 12 cc of 40 per cent formalin, 30 cc of 95 per cent alcohol, 2 cc of glacial acetic acid, and 60 cc of distilled water). The liver, kidneys, spleens, and muscles were gradually dehydrated with alcohol solutions. After the drying process was completed, the samples were placed in celloidin for a period of six months. The bones, after dehydration, were treated with Von Ebner's fluid (500 cc of 95 per cent ethyl alcohol, 100 cc of water, 2.5 g sodium chloride, and 5.5 cc of concentrated hydrochloric acid). As soon as the bones were decalcified, they were placed in celloidin for a period of six months. Sections were cut  $12\mu$  in thickness and were stained with hematoxylin.

In an anemic animal morphological changes would naturally be sought in the hematopoietic tissues. Commonly these are fatty changes, and degeneration in the kidneys and liver, together with some necrosis. Hematopoietic centers appear in the liver and spleen, while fat tends to disappear from the bone marrow, being replaced by erythroblastic tissue.

All the tissues in this study appeared to be normal, and very similar for both groups of rats. In the spleens the amount of lymphoid tissue was about the same, and centers of hematopoiesis could be identified. In the bone marrow the fat content was about the same for both groups, and the cell types did not vary appreciably—thus indicating normal cellular activity.

These observations indicate that the low-iron diet did not differ in any marked respect from the meat diet in its ability to nourish the tissues involved in blood formation, and that any iron impoverishment caused by the lower intake of iron on this diet was not sufficiently

severe to cause pathological changes in these tissues in the course of 15 weeks' feeding.

#### SUMMARY AND CONCLUSIONS

A comparison was made of the effects of 2 diets—1 low in iron, and 1 high in iron—on the growth, the fasting metabolism, the blood composition, and the reproduction of the albino rat; and also on the histology of the liver, the kidney, the spleen, the skeleton, and the muscle of this species.

The iron-poor diet contained 0.001 per cent iron, the source of which was dried skim-milk powder. The iron-rich diet contained 0.002 per cent iron, the source of which was beef muscle and dried skim-milk powder.

An average daily intake of 0.06 mg of iron from skim-milk powder was found to be sufficient for normal growth. No significant difference was found in the average rate of growth of the experimental subjects on the two diets in either the first or the second generation.

The average of the respiratory quotients, after 16 to 50 hours without food, was 0.75 for the rats on both diets. The average daily heat produced was 15.56 and 15.34 calories per 100 g of body weight ( $W^{2/3}$ ) and 967 and 953 calories per square meter of body surface ( $S = KW^{2/3}$ ), on the low-iron and high-iron diets, respectively.

The average number of erythrocytes and of leucocytes per cubic millimeter of blood was approximately the same for rats confined to these diets during the first and also during the second generation.

The average number of grams of hemoglobin per 100 cc of blood was also normal on both diets for two generations of feeding. The rats receiving the high-iron diet during the first generation had an average of 2.07 g more of hemoglobin per 100 cc of blood than had those receiving the low-iron diet. This difference was found to be statistically significant, but the rats on the low-iron diet could not be said to be anemic.

It was shown that the low-iron diet was inadequate for the most successful reproduction, and that this effect was more prominent during the second than during the first generation. The females of the first generation gave birth to 6.9 and 8.4 young per litter, on the average, and weaned 65.0 and 84.5 per cent of the young while confined to the iron-poor and the iron-rich diets, respectively. The females of the second generation gave birth to 6.9 and 8.3 young per litter, on the average, and weaned 34.8 and 79.0 per cent of the young while confined to the iron-poor and the iron-rich diets, respectively.

Sterility and cannibalism were observed among the second generation females which received the low-iron diet.

No pathological conditions were found as to histology of the tissues of the liver, kidney, spleen, bone, and muscle that could be considered as effects of the low-iron diet.

When the protein required for growth was provided by skim-milk powder, in a diet which satisfied the energy and the vitamin requirements, normal growth resulted, even though the iron content of the diet was low; the nutrition of the blood, and of the tissues studied and the rate of energy metabolism were normal; but this low-iron diet did not satisfy the requirements for normal reproduction. These requirements were provided, however, by a similar diet containing additional iron as supplied by beef muscle.

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## LYDELLA NIGRIPES AND L. PINIARIAE, FLY PARASITES OF CERTAIN TREE-DEFOLIATING CATERPILLARS<sup>1</sup>

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### INTRODUCTION

For a number of years the United States Bureau of Entomology has been collecting the European parasites of the gipsy moth (*Porthetria dispar* L.) and the brown-tail moth (*Nygmia phaeorrhoea* Don.) for liberation in New England. The tachinid fly *Lydella nigripes* Fallén is a minor parasite of both these pests. Its mode of life is similar to that of *Compsilura concinnata* Meig., which became established in the United States in 1907. *C. concinnata* has proved highly successful in its new environment, and it was believed that *L. nigripes* might do as well if once established. Accordingly, in 1928 and 1929, a special attempt was made to secure large numbers of the species from Europe. At that time it was noted from the literature that the species overwintered as a first-instar larva in the pupa of the pine geometrid (*Bupalus piniarius* L.). A heavy outbreak of *B. piniarius* in north-western Poland at Chorinskimlyn seemed to present an excellent opportunity to rear the parasite, and 100,000 host pupae were collected in the spring of 1928. Recoveries were disappointingly small. Only about 1,800 puparia were secured. These were all sent to the laboratory of the Bureau of Entomology at Melrose Highlands, Mass., for colonization. In 1929 the work was continued on a larger scale at Zwierzyniec, near Lwow, Poland, and about 57,000 puparia were obtained.

The same year biological investigations of the species were started. The flies reared from *Bupalus piniarius* behaved in a manner very different from what had been expected. They practically refused to attack either gipsy or brown-tail moth larvae, in spite of the fact that specialists in Tachinidae considered flies reared from the three hosts to be identical. Investigations of that year and subsequent ones have shown that flies reared from *B. piniarius* actually have very different habits and life histories from those of flies reared from *Porthetria dispar* or *Nygmia phaeorrhoea*. It was possible, though, to show that flies reared from *P. dispar* and *N. phaeorrhoea* were identical. These

<sup>1</sup> Received for publication Nov. 5, 1932; issued July 1933. This study was conducted under the direction of C. W. Collins, in charge of the U. S. Bureau of Entomology laboratory at Melrose Highlands, Mass., at its European substation, Budapest, Hungary, during 1929, 1930, and 1931.

<sup>2</sup> The writer wishes to express his gratitude to Wendell F. Sellers for descriptions of the adult flies, to Ferencz Mihályi for drawings of the adult and for laboratory assistance, to Josef Ujhelyi, R. C. Brown, W. E. Ripper, and Anton Linke for help in making collections in Europe, and to R. T. Webber, T. H. Jones, and Mrs. P. B. Dowden for many helpful suggestions in the preparation of the manuscript.

reared from *P. dispar* and *N. phaeorrhoea* are the true *Lydella nigripes* Fallén, while those from *B. piniarius* are *Lydella piniariae* Hartig.

This paper presents a study of the bionomics of these two species of *Lydella* and a comparison of their life histories, and shows their close relationship. The true *L. nigripes* has been studied in particular because of its importance as a parasite of the gipsy and brown-tail moths. This species has also been compared briefly with *Compsilura concinnata*.

#### REVIEW OF LITERATURE

Although a full account of the biology of *Lydella nigripes* has never been published, references to the species are common in the literature. These references do not distinguish between *L. nigripes* (*Dexodes nigripes*) and *L. piniariae*, but when they give *Porthetria dispar*, *Nygmia phaeorrhoea*, or *Bupalus piniarius* as the host species parasitized, there is little question as to the identity of the parasite.

##### LYDELLE NIGRIPES

In 1908 Townsend (17)<sup>3</sup> noted briefly the manner in which *Lydella nigripes* deposited living maggots beneath the skin of host caterpillars and its rapid development in larvae of *Euproctis chrysorrhoea* L. (= *Nygmia phaeorrhoea*) and *Heterocampa leucostigma* S. and A. Pantel (10) in 1910 stated that it belonged to the same group as *Compsilura concinnata*, which he described in some detail. Howard and Fiske in 1911 (7) and Burgess and Crossman in 1929 (3) briefly noted the life history of the species. They also gave summaries of the number of *L. nigripes* puparia recovered from rearings of European *Nygmia phaeorrhoea* and *Porthetria dispar*, and the number of flies liberated in the United States, with a discussion of the possibility of the species becoming established in North America.

##### LYDELLE PINIARIAE

In 1914 Plotnikov (11), in a paper on the biology of *Bupalus piniarius* and its parasites, considered *Lydella nigripes* (= *piniariae*) one of the most important parasites of that species. He described its life history in this host briefly, figuring and describing the first larval instar in detail. In 1921 Baer (1) called attention to the polyphagous habits of the species, and emphasized its importance as a parasite of *B. piniarius*. Seitner (12) mentioned it in 1921, and Sitowski (13) in 1922, Eidmann (5) in 1926, Czerwinski and Kuntze (4) in 1930, and Steiner (14) in 1931 briefly sketched its life history and gave valuable information regarding its effectiveness as a parasite of *B. piniarius*. In 1931 Escherich (6, v. 3, p. 463-569) reviewed these references in a comprehensive study of *B. piniarius*.

#### DISTRIBUTION AND HOST RELATIONSHIPS

*Lydella nigripes* is common throughout Europe. Rearings of gipsy and brown-tail moth material in connection with the work of the United States Bureau of Entomology show recoveries from Spain, France, Italy, Belgium, Holland, Germany, Czechoslovakia, Switzerland, Austria, Hungary, Yugoslavia, Poland, and Russia. Probably its range is even greater.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 994.



*Lydella pinariae* is also common throughout Europe, if we may judge from the fact that *L. nigripes* is recorded as a parasite of *Bupalus piniarius* in France, Germany, Austria, Czechoslovakia, Denmark, Poland, and Russia.

A remarkably large number of hosts for *Lydella nigripes* are mentioned in European literature. Since no distinction is made between *L. nigripes* and *L. pinariae*, the references may, of course, refer to either one. The writer's experiments showed, however, that *L. nigripes* was exceptionally polyphagous, whereas *L. pinariae*, although given almost as many host larvae to attack, was able to complete development only in *Bupalus piniarius*, *Abrostola tripartita* Hufn., and *Tortrix dumetana* Freyer. It has therefore seemed advisable to make two host lists. The first gives records from the literature. The second gives records which were made in connection with this study from larvae parasitized in the laboratory and from collected larvae that had been parasitized under natural conditions in the field.

#### HOSTS OF *LYDELLA NIGRIPES* AS RECORDED IN THE LITERATURE

##### Lepidoptera:

*Acronicta rumicis* L. (1).  
*Agrotis candellarum* Stmgr. (2).  
*Aporia crataegi* L. (8).  
*Acosmetia caliginosa* Hbn. (2).  
*Bupalus piniarius* L. (2).  
*Brephos parthenias* L. (9).  
*Calymnia trapezina* L. (9).  
*Cucullia artemisiae* Hufn. (9).  
*Cucullia asteris* Schiff. (2).  
*Cucullia scrophulariae* Cap. (9).  
*Deilephila euphorbiae* L. (2).  
*Dendrolimus pini* L. (1).  
*Dryobota protea* Bkh. (9).  
*Euproctis chrysorrhoea* L. (1).  
*Eurrhpara urticata* L. (2).  
*Heliothis scutosa* Schiff. (2).  
*Hematurga atomaria* L. (5).  
*Hybernia* sp. (2).  
*Larentia badiata* Hbn. (1).  
*Leucania pallens* L. (1).

##### Lepidoptera—Continued

*Lymantria dispar* L. (1).  
*Mamestra pisi* L. (2).  
*Miana literosa* Haworth (2).  
*Ortholitha cervinata* Schiff. (2).  
*Phragmatobia fuliginosa* L. (2).  
*Plusia gamma* L. (2).  
*Polia oleracea* L. (19).  
*Porthesia similis* Fuess. (2).  
*Tapinostola elymi* Treit. (2).  
*Tephroclystia innotata* Hufn. (9).  
*Tephroclystia virgaureata* Dbl. (2).  
*Thaumetopea pinivora* Treit. (2).  
*Vanessa io* L. (2).  
*Vanessa polychloros* L. (2).  
*Vanessa urticae* L. (2).  
*Yponomeuta padellus* L. (1).

##### Hymenoptera:

*Lophyrus* sp. (2).  
*Nematus ribesii* Scopoli (2).

#### HOSTS OF *LYDELLA NIGRIPES* DETERMINED FROM REARINGS IN EUROPE

##### LARVAE PARASITIZED UNDER NATURAL CONDITIONS IN THE FIELD

##### Lepidoptera:

*Abrostola tripartita* Hufn.  
*Arctia caia* L.  
*Malacosoma neustria* L.  
*Nygmia phaeorrhoea* Don.  
*Porthesia similis* Fuess.

##### Lepidoptera—Continued

*Porthetria dispar* L.  
*Polia trifolii* Rott.  
*Thaumetopea processionea* L.  
*Vanessa urticae* L.

##### LARVAE PARASITIZED IN THE LABORATORY

##### Lepidoptera:

*Abrostola triplasia* L.  
*Acronicta megacephala* Fab.  
*Acronicta rumicis* L.  
*Acronicta tridens* Schiff.  
*Agrotis c-nigrum* L.  
*Calophasia casta* Bkh.  
*Cucullia asteris* Schiff.  
*Eriogaster lanestris* L.  
*Heliothis dipsacea* L.

##### Lepidoptera—Continued

*Oryzestea geographica* Fab.  
*Phalera bucephala* L.  
*Polia brassicae* L.  
*Polia oleracea* L.  
*Synopsis sociaria* Hbn.  
*Vanessa io* L.

##### Hymenoptera:

*Trichiocampus viminalis* (Fall.).

*Lydella nigripes* also completed development on two lepidopterous larvae attacked at the gipsy moth laboratory at Melrose Highlands, Mass., namely, *Alypia octomaculata* Fab. and *Heterocampa leucostigma* S. and A.

Under natural conditions in the field *Lydella pinariarum* parasitized larvae of *Bupalus piniarius* L. (Lepidoptera) and in the laboratory it parasitized larvae of *Tortrix dumetana* Freyer and *Abrostola tripartita* Hufn. (both Lepidoptera).

With the two exceptions noted all attempts to rear *Lydella pinariarum* at the laboratory were unsuccessful. Judging from the long list of successful laboratory rearings of *L. nigripes*, one feels that the great majority of hosts given in the literature actually belong to this species.

### ECONOMIC IMPORTANCE

#### LYDELLA NIGRIPES

The early work on *Lydella nigripes* done at the Melrose Highlands laboratory indicated that this insect was important as a parasite of the brown-tail moth in Europe. It was reared in small numbers from shipments of larvae in 1906, 1907, and 1911, and in fairly satisfactory numbers in 1909. A total of 5,212 was secured during this period. At that time it was rarely reared as a parasite of the gipsy moth.

Since the foreign work was resumed in 1922 no large collections of *Nygmia phaeorrhoea* have been made, but *Lydella nigripes* has at times been of some importance as a parasite of *Porthetria dispar*. Table 1 gives the number of puparia recovered.

TABLE 1.—*Recovery of Lydella nigripes and total tachinid puparia from collections of Porthetria dispar in Europe, 1926 to 1931, inclusive*

Year	Place of collection	<i>P. dispar</i> larvae col- lected	<i>L. nigripes</i> puparia re- covered	Total tach- inid pupa- ria recov- ered
1926	Galgamacs, Hungary	225,000	2,784	58,398
	Baja, Hungary	300,000	870	80,451
	Doboz, Hungary	250,000	1,023	11,965
	Olaszliszka, Hungary	400,000	1,847	40,971
1927	Simontornya, Hungary	188,000	1,734	35,537
	Rembertow, Poland	200,000	183	51,566
	Moscenica, Yugoslavia	150,000	293	11,388
	Vecs, Hungary	230,000	77	42,734
1928	Novoselec Kriz, Yugoslavia	178,000	25	44,756
1929	Vecs, Hungary	189,000	0	14,224
	Platicevo, Yugoslavia	239,000	0	14,415
	Jánk, Hungary	320,000	10	37,289
	Oszro, Hungary	238,000	5	95,819
1930	Csepel, Hungary	4,000	0	641
	Platicevo, Yugoslavia	250,000	0	28,374
	Galgamacs, Hungary	331,000	10	11,514
	Oszro, Hungary	406,000	540	93,199
1931	Oberpullendorf, Austria	230,000	31	73,108

In 1927, 1930, and 1931 a few *Nygmia phaeorrhoea* larvae were collected. A record of the *Lydella nigripes* reared is given in Table 2.

TABLE 2.—*Lydella nigripes* puparia reared from collections of *Nygmia phaeorrhoea* in Europe, 1927, 1930, and 1931

Year	Place of collection	<i>N. phaeorrhoea</i> larvae collected	<i>L. nigripes</i> puparia reared
1927	Budapest, Hungary	250	44
	do	1,565	10
	Jánk, Hungary	126	55
1930	Oszro, Hungary	83	0
	Platicevo, Yugoslavia	300	0
	Linz, Austria	1,200	18
1931	Budapest, Hungary	1,500	16
	Oberpullendorf, Austria	1,000	48

#### LYDELLE PINIARIAE

*Lydella piniariae* is much more important in controlling *Bupalus piniarius* than is *L. nigripes* in controlling the gipsy moth and the brown-tail moth. It is generally present in comparatively small numbers in the first year of a *Bupalus* infestation, but after that it increases rapidly. Plotnikov (11) notes it as parasitizing 12 per cent of the larvae and Sitowski (13) records it as parasitizing 25 per cent.

The writer's rearings are recorded in Table 3.

TABLE 3.—*Recovery of Lydella piniariae* puparia from collections of *Bupalus piniarius* in Europe, 1928, 1929, and 1930

Year	Place of collection	Overwintering <i>B. piniarius</i> pupae collected	<i>L. piniariae</i> puparia recovered	Parasitization by <i>L. piniariae</i>
		Number	Number	Per cent
1928	Chorinskimlyn, Poland	120,000	1,836	1.5
1929	Zwierzyniec, Poland	735,000	57,838	7.8
1930	Gora, Poland	3,000	600	20.0

#### DESCRIPTION

The adult flies of *Lydella nigripes* and *L. piniariae* have been separated by W. F. Sellers, of the gipsy moth laboratory. Papers by Thompson (15) and by Thompson and Thompson (16) have been very helpful in preparing the descriptions of the immature forms.

#### ADULT

A description of *Lydella nigripes* (fig. 1) by Lundbeck (9, pt. 7, p. 299) in its main essentials is an excellent description of the two forms treated in this paper, but it does not serve to differentiate them. According to Sellers, however, the two species can be readily distinguished by the relationship of the front at its narrowest point to the head at its widest part. The ratio was secured by dividing the smaller measurement by the larger. *L. nigripes* has an average range of from 0.224 to 0.245 mm in the males, and from 0.293 to 0.308 mm in the females, while *L. piniariae* has an average range of from 0.274

to 0.286 mm in the males, and from 0.324 to 0.333 mm in the females. Since the two forms are readily separated and do not show a natural tendency to interbreed, it is believed that they have become sufficiently stabilized to be considered as having a more specific than racial relationship.

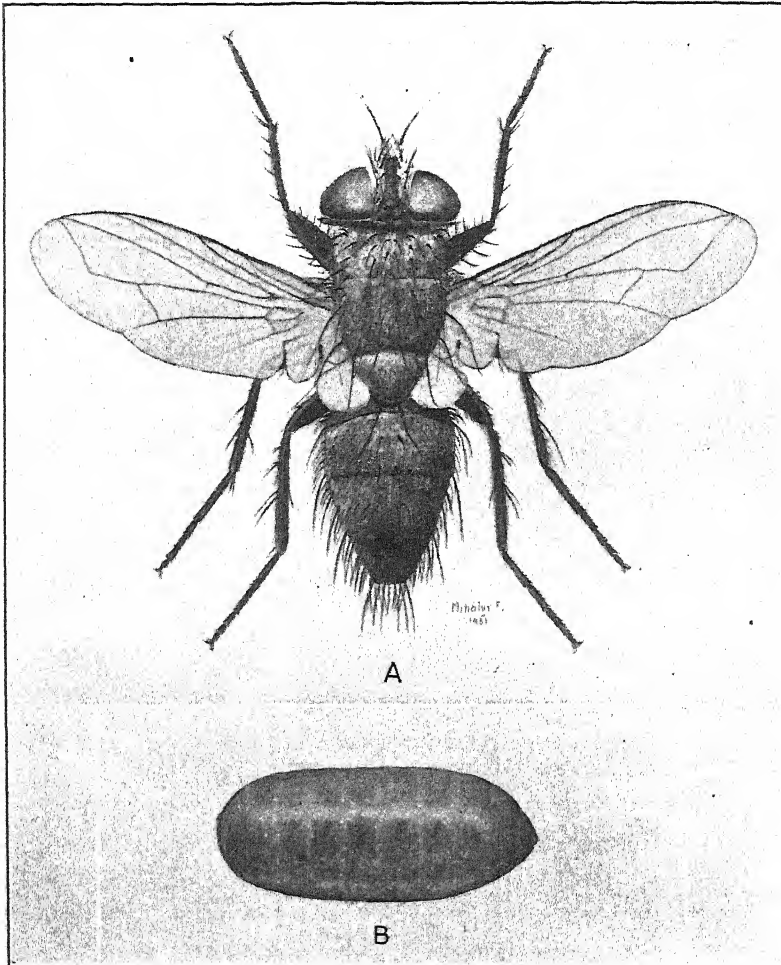


FIGURE 1.—*Lydella nigripes*: A, Adult female; B, puparium

Sellers found that both forms were included in the type series of both species. He concludes that the bulk of the synonymy probably represents the form reared from *Porthetria dispar* and numerous other hosts—i. e., the one with the smallest ratio—and that this is the true *Lydella nigripes* Fallén, while the form reared from *Bupalus piniarius* is *L. piniariae* Hartig. In Meigen's collection at Vienna, Austria, there are two cotypes of *nigripes* Fallén. Sellers has designated the male as the true *nigripes*; the female is *Lydella piniariae* Hartig.

## IMMATURE STAGES

## LYDELLA NIGRIPES

## THE EGG

When the egg enters the uterus it measures about 0.66 mm long and 0.21 mm wide. It has the form of an ellipse, but is rounded off at each end. The chorion is very thin and transparent over the entire surface except in the terminal micro-

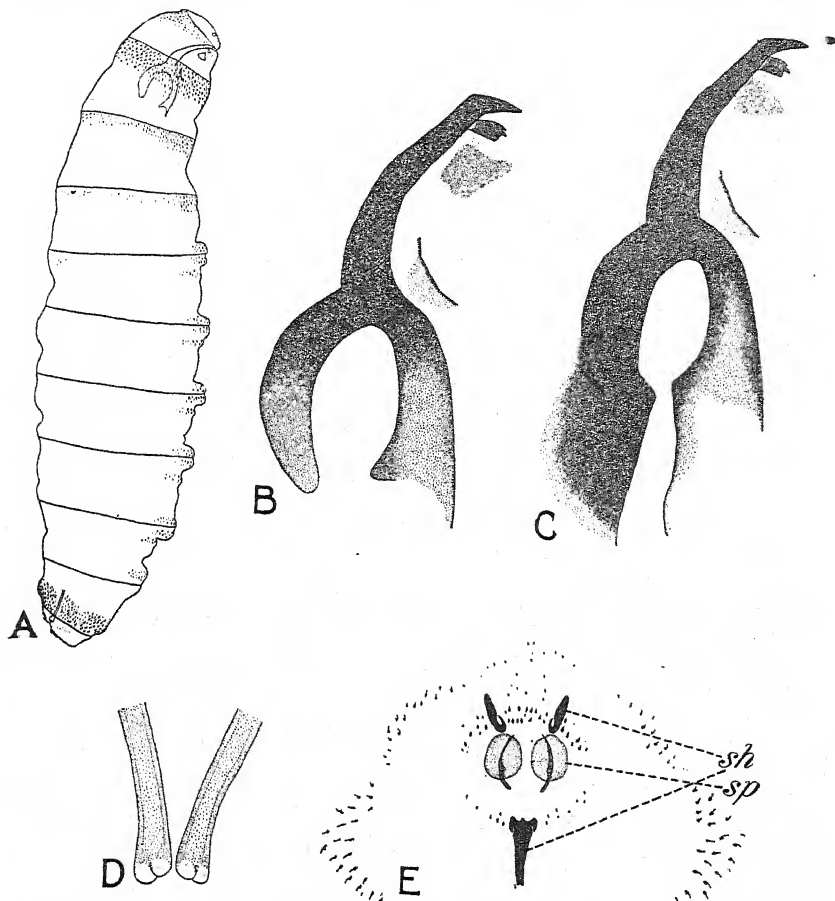


FIGURE 2.—*Lydella nigripes*, first larval instar: A, larva, lateral aspect; B, buccopharyngeal armature of freshly larviposited specimen; C, buccopharyngeal armature of 4-day-old specimen; D, posterior spiracle chambers; E, posterior spiracles, *sp*, showing cuticular armature of eleventh segment, and stigmatic hooks, *sh*.

pyle region, where it is somewhat thicker and roughened. With the development of the embryo to a fully formed larva, the chorion stretches considerably.

## FIRST-INSTAR LARVA

The first-instar larva of *Lydella nigripes* (fig. 2, A) is cylindrical, tapering slightly at both extremities. Freshly deposited specimens are from 0.90 to 1.20 mm long and from 0.30 to 0.35 mm wide. Full-grown first-instar larvae average 2 mm in length and 0.75 mm in width. The body is composed of the pseudo-cephalon, bearing the antennal and sensorial organs, and 11 body segments. The cuticle is colorless and transparent, so that the tracheae and portions of the diges-

tive system are plainly visible. The pseudocephalon is unarmed, but on every other segment the cuticle bears rows of minute spines. The first three anterior (thoracic) segments are completely encircled anteriorly. The fourth (first abdominal) segment has a complete anterior band of spines, which are quite scattered in the pleural region. Ventrally this band is divided into two groups which are separated longitudinally by a narrow but well-defined bare space. This segment also bears a small group of spines ventrally on its posterior border. On segment 5 the encircling anterior band is absent. There are a few very small dorsal spines, but none in the pleural region. Ventrally there are two narrowly separated groups on the anterior border and a small group on the posterior border as on segment 4. The arrangement of spines on segments 6 to 9 is practically identical with that on segment 5 except that the few dorsal spines are lacking. Segment 10 bears no anterior spines, but it has a completely encircling band on its posterior border. The eleventh segment has no bands of spines comparable to those of the other segments, but it supports the three curious and interesting stigmatic hooks (fig. 2, E) which are characteristic of this type of larva. Two of them lie just anterior to the anal spiracles, and are simple strongly curved hooks about 0.015 mm long. They are directed forward. The third, which measures 0.02 mm, occupies a position midway between the first two but posterior to the anal spiracles. At its anterior extremity it is divided into two hooks that point backward. These hooks are used in this species, as Pantel (10) described them in *Compsilura concinnata*, to attach the larva to the intestine of the host. Besides these hooks there are a few spines on the pleural portion of the eleventh segment and also surrounding the spiracular openings.

The arrangement of the cuticular spines in rows is indicated in Figure 2, A. Those on the anterior borders of the segments point backward, while those on the posterior borders point forward. The rows of spines have no absolutely definite arrangement, but for the most part each spine in one row occurs opposite the space between two spines in the row preceding it.

The fact that each segment from the fourth to the eighth, inclusive, has a row of ventral spines on the posterior border followed closely by two rows of spines on the anterior border of the following segment gives the larva the superficial appearance of having segments bearing three closely situated rows of spines ventrally. This appearance is so deceptive that Plotnikov (11) described the larva in this manner, but he did not study the later larval instars, which show the true relation more clearly. The reason the location of the actual segmental division is so hard to define is that the spines occur on the pseudopodia or false feet, which are formed by flesh growing internally between the segments on the venter, thus pushing the cuticle out.

The buccopharyngeal armature of the freshly emerged larva is shown in Figure 2, B. It is about 0.18 mm long. The median tooth is a single piece which is divided at the basal region into two pairs of basal wings. There are two pairs of lateral plates at the apex of the armature and a single salivary gland plate at the base of the median tooth. As the larva grows the form of the armature is considerably changed owing to the progressive sclerotization<sup>4</sup> which takes place in the pharyngeal cuticle surrounding the basal plates. (Fig. 2, C.)

The larva is metapneustic. The two posterior spiracles open on the dorso-pleural portion of the last abdominal segment. The spiracular chambers (fig. 2, D) are about six times as long as wide and measure 0.06 mm in length. From these chambers a well-developed tracheal system arises in two main tracheal trunks, which lie dorsally in the body cavity.

The only sensory organs observed in the larva are located on the pseudocephalon. There are four pairs, one element of each lying on opposite sides of the mouth opening. The most conspicuous are the antennal organs, which are circular, slightly protuberant, and measure 0.0075 mm in diameter. Just below them are the maxillary organs, composed of a group of about 10 minute circular points very slightly raised from the surface of the cuticle. Just below the antennae are two minute organs and just below each maxillary group another minute organ is found. The last two groups are so small that they are impossible to find in most specimens. A very delicate fold surrounds the antennal and maxillary organs, thus raising the group slightly from the rest of the cuticle.

<sup>4</sup>"Sclerotization" as used in this paper refers to the darkening and hardening of the area.

## SECOND-INSTAR LARVA

As the first-instar larva approaches maximum growth the organs of the second instar can be plainly seen forming under the skin. The old skin finally splits just above the anal spiracles and larval movement forces it over the head. The second-instar larva (fig. 3, A) is considerably larger than the first-instar larva, measuring about 3 mm long and 1 mm wide when first formed and about 5.33 mm long and 1.5 mm wide when full grown. It has the same form as the first-instar larva and the cuticle is still colorless and transparent. The cuticular armature is heavier and more conspicuous. The rows are now formed of lineal groups of from 4 to 12 spines. These groups are quite distinctly separated from one another

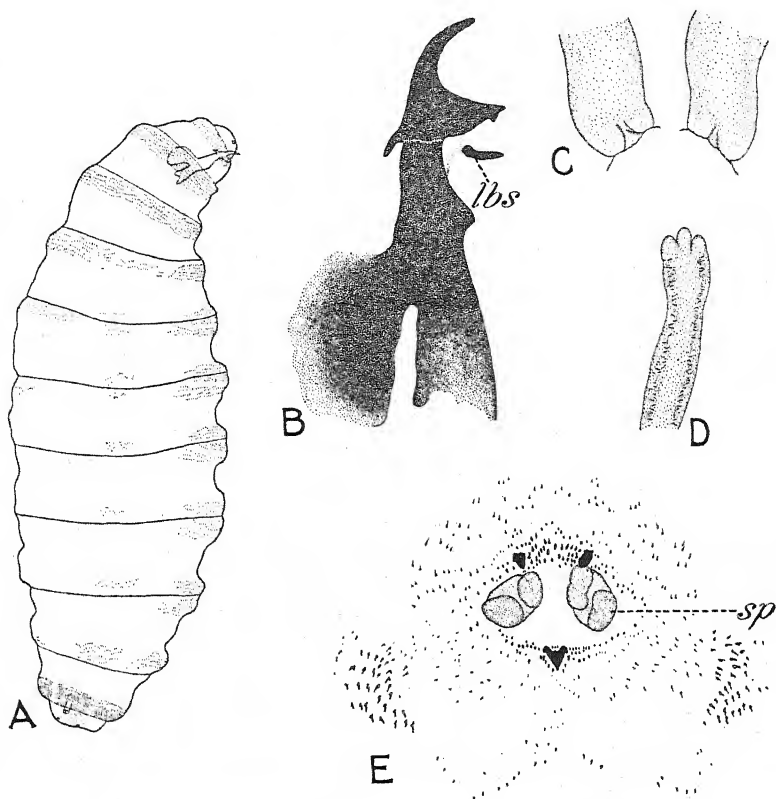


FIGURE 3.—*Lydella nigripes*, second larval instar: A, larva, lateral aspect; B, buccopharyngeal armature showing labial sclerite, *lbs*; C, posterior spiracle chambers; D, anterior spiracle chamber; E, posterior spiracles, *sp*, showing surrounding cuticular armature

and curved so that the rows become indistinct, but the arrangement is much the same as in the first instar. The pseudoccephalon bears no spines. The first three segments have anterior bands of spines only. The fourth segment bears a completely encircling band of spines on its anterior border, but in the pleural region the spines are rather scattered. This segment bears spines on its posterior border also. On the fifth segment the anterior band is distinctly broken by a bare area on each side of the small pleural group. In the ventral region this band is made up of two groups of spines separated by a narrow longitudinal bare strip. Each of these areas has about four rows of spines, but the anterior one is nearly uniform in width while the posterior one is considerably longer and much narrower, or is even broken at the center. On the posterior border of this segment there is a small group of spines in the ventral region and also in the pleural region. Segments 6 to 9 are much the same as segment 5. On segment 6 the dorsal spines on the anterior border have practically disappeared, and the anterior pleural spines

are reduced. On segments 7, 8, and 9 the spines on the anterior borders are present only ventrally, where they diminish slightly in numbers proceeding posteriorly. The spines on the posterior borders, however, increase in numbers slightly on the seventh and eighth segments, and on the ninth segment the posterior pleural group is quite prominent and a few posterior dorsal spines are present. The tenth segment bears a complete band on its posterior border. In this band there is an almost bare area both dorsopleurally and ventropleurally. The eleventh segment bears no spines comparable to those of the other segments. The stigmatic hooks are situated as before, but in this instar each is an aggregate of hooks rather than a single piece. The anterior pair have two or three forward-pointing hooks, which are fused at their bases into a small, irregularly shaped area. The posterior element is made up of five or six hooks which point backward. The fusion of their bases forms a V-shaped sclerite. (Fig. 3, E.) On the sides of this segment there are a few rows of spines and scattered over the whole segment there are small spines.

The arrangement of the cuticular armature is shown in Figure 3. The direction of the spines is the same as in the first instar. The pseudopodia are more pronounced.

The buccopharyngeal armature (fig. 3, B) has undergone considerable change. Instead of a single median tooth, there is a pair of deeply curved anterior hooks which are separated from the basal region by a narrow incision. Lateral plates at the apex of the anterior hooks are no longer present. Just below the posterior margins of the hooks two small sclerites can be distinguished. These support the labium. When the structure is viewed in its ventral aspect, the labium can be distinguished between the bases of the anterior hooks, posterior to the labium the hypopharyngeal plate, and below that the epipharyngeal plate. Both of these plates are essentially the same in the third instar, and are described in more detail in the next section. There is no progressive sclerotization in the basal region of the second-instar buccopharyngeal armature. Immediately after the first-instar larval skin is shed only the anterior portions of the hooks are sclerotized, but sclerotization of the rest of the armature takes place very rapidly. Usually the edge of the dorsal wing is not fully pigmented.

The second-instar larva is amphipneustic. The anterior spiracles (fig. 3, D) open on the posterior border of segment 1 in the pleural region. Each has three respiratory papillae and a lightly colored forechamber leading to the tracheal tubes. The posterior spiracles (fig. 3, C) open on the dorsopleural portion of the eleventh segment. The spiracular chambers are about twice as long as wide. The tracheal system is much heavier than in the first instar.

The sensory organs on the pseudocephalon have about the same appearance as in the first instar, but they are larger and more conspicuous. In this instar there are also two pairs of sensory organs on the eleventh body segment. They are tiny fingerlike projections; each element of one pair is situated on each side of the posterior spiracular hook, and each element of the other pair is placed just inside one of the anterior spiracular hooks.

#### THIRD-INSTAR LARVA

The third-instar larva (fig. 4, A) has the same form as in the preceding instar. Its size varies greatly. When first formed it is about 6 mm long and 2 mm wide, when full grown from 7 to 9 mm long and from 2 to 2.50 mm wide. The cuticle is colorless and transparent and armed, as before, with tiny spines, but there is considerable variation in their pigmentation so that in some specimens they are more difficult to see than in others. The spines are again arranged in curved lineal groups, which are even more distinctly separated than in the second instar.

The pseudocephalon is unarmed. Segments 1 to 3 have anterior bands of spines only. On segment 4 the anterior band is interrupted in the pleural region, while ventrally it is widened into two groups slightly separated from each other by a narrow longitudinal bare area. On the posterior border of this segment there is a small ventral and a small pleural group of spines. On segment 5 the anterior band of spines is more distinctly broken in the pleural region, while ventrally the two groups are distinctly separated. The anterior of these groups is rather short but of an even width throughout. The posterior group is longer but very narrow or broken at the center. The posterior border of this segment is armed similarly to segment 4, but the pleural group is longer. Segments 6 to 9 are armed similarly to segment 5, but proceeding posteriorly the spines on the anterior borders of the segments become less numerous and those on the posterior borders more plentiful. On segment 9 the posterior border is completely encircled. On segment 10 the band on the posterior border is complete and heavy throughout, although there is



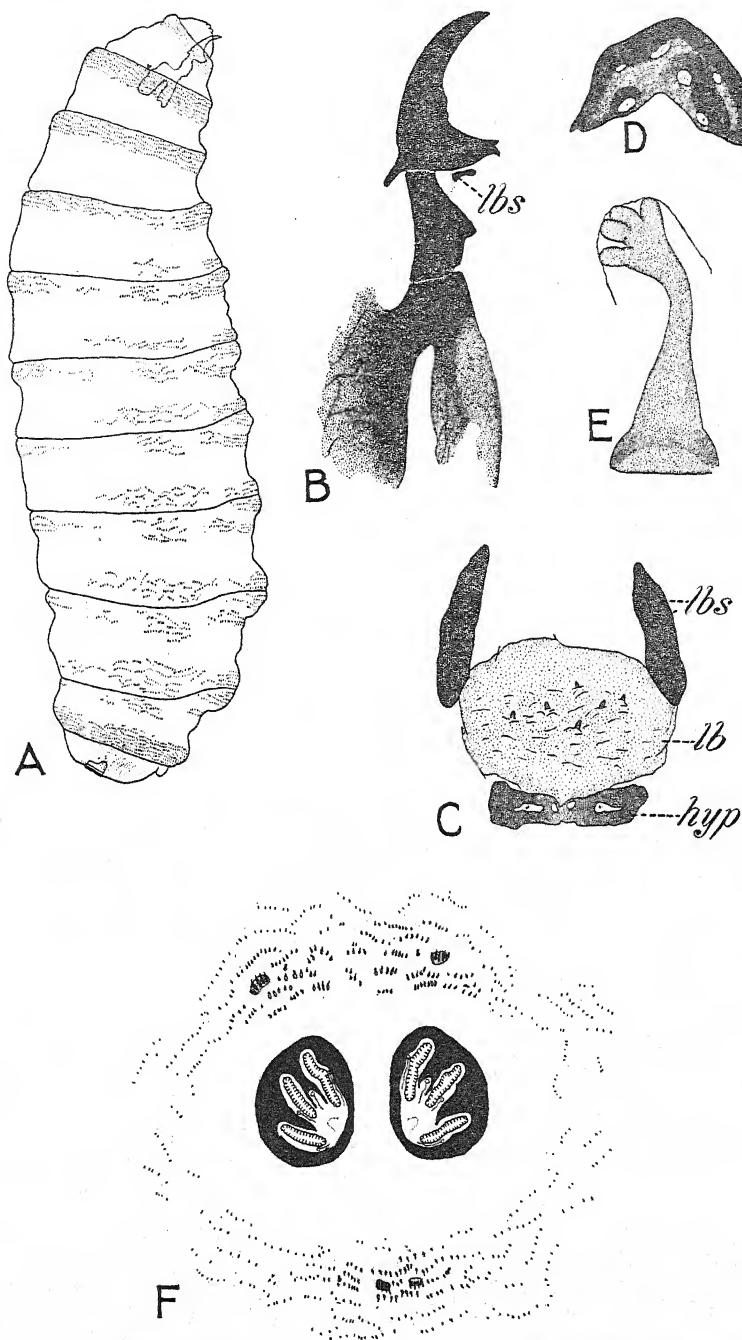


FIGURE 4.—*Lydella nigripes*, third larval instar: A, Larva, lateral aspect; B, buccopharyngeal armature, showing labial sclerite, *lbs*; C, hypopharyngeal plate, *hyp*, labial sclerite, *lbs*, and labium, *lb*; D, epipharyngeal plate; E, anterior spiracle chamber; F, posterior spiracles, showing surrounding cuticular armature

a narrow bare area in the dorsopleural region. Segment 11 bears the heavy posterior spiracles on its upper caudal aspect (fig. 4, F), which are surrounded by rather stout spines. The stigmatic hooks are lacking, but in the positions that they occupy in the earlier instars appear little clusters of three or four spines with their bases fused together to form a pigmented plate. Often there are two such groups posteriorly. Aside from the spines around the spiracles there is a conspicuous group of spines that are directed away from the spiracles.

The direction of the spines is again the same in this instar, and the pseudopodia are even more pronounced than in earlier instars.

The form of the buccopharyngeal armature (fig. 4, B) does not differ greatly from that observed in the preceding instar, but an articulation exists between the base of the intermediate region and the basal plates. The sclerites which support the labium are again apparent, just below the posterior margin of the anterior hooks. Figure 4, C, shows the inner surface of the labium with its supporting sclerites on each side, and just posterior to it is the hypopharyngeal plate. This plate varies considerably in different specimens. Sometimes the two openings on each side are fused together, and sometimes the two sides are separated by a suture at the middle, although the general form is that given in the figure. The epipharyngeal plate (fig. 4, D) is found just dorsad of the hypopharyngeal plate near the center of the space formed by the two sides of the intermediate wings of the buccopharyngeal armature. It is even more variable in structure than the hypopharyngeal plate. Sometimes it has a very sharp angle at the apex and often some of the openings are not visible. The figure is taken from a fairly representative specimen. Progressive sclerotization of the armature takes place as in the second instar.

In the third instar the larva is again amphipneustic. The anterior spiracles (fig. 4, E) have three or four papillae. The lightly sclerotized tube leading to the tracheae is narrowed behind the respiratory papillae, and then gradually widens out into a cup-shaped base. Each of the posterior spiracles (fig. 4, F) possesses three respiratory slits surrounded by a heavily pigmented horny area. The spiracular chambers are short and somewhat longer on the lower than on the upper side. The tracheal system is conspicuous.

The sensory organs on the pseudocephalon are the same as in the second instar except that they are larger and more pronounced. Those on the eleventh segment also are the same as in the second instar except that they are larger.

#### THE PUPARIUM

The puparia (fig. 1, B) vary greatly in size, but they average about 7 mm long and 2.5 mm wide. When first formed they are yellowish in color, but in most cases they quickly change to a dark reddish brown. The surface is smooth, although the former segmental lines are faintly visible, and the minute cuticular armature of the third-instar larva can be distinguished under the microscope. The anterior extremity is rounded, the posterior extremity subconical. At the apex there is a small but pronounced rugose elevation which has a longitudinal depression. Just dorsad and on each side of this depression are the rather inconspicuous posterior spiracles. They lie flat against the surface and very close to each other, being separated by no more than one-half their diameter. The anterior spiracles are slightly raised and barely visible to the naked eye. The anus can be seen on the venter at the juncture of the tenth and eleventh segments.

The respiratory apparatus of the pupa has the form shown in Figure 5, C. The prothoracic cornicles are horny structures which protrude from the surface of the puparium just in front of the posterior margin of segment 4. At their distal extremities there are about 12 small openings. The internal spiracle is nearly circular. Its surface is covered with a large number of tiny papillae, which are arranged in double rows radiating out from the central portion.

#### LYDELLA PINIARIAE

The description of the immature instars of *Lydella nigripes* applies equally well to the same forms of *L. piniariae* with the exception of two minor differences observed in the buccopharyngeal armature of the first-instar larva. A comparison of this structure in the two

species shows that the apex of the anterior hook in *L. nigripes* (fig. 2, B) is distinctly more sharply angled than is the case in *L. piniariae* (fig. 5, B). Occasionally specimens of *L. nigripes* show a somewhat weaker angle, which makes it difficult to separate them from *L. piniariae*, but most specimens are distinct. Another interesting difference between the two species is that *L. nigripes* almost invariably shows a progressive sclerotization of the basal lobes of the buccopharyngeal armature after it is 3 or 4 days old, whereas this never takes place in *L. piniariae*. Occasionally a larva of *L. nigripes* is stunted in growth and the progressive sclerotization does not occur, and at times there is a slight further sclerotization in *L. piniariae*. Nevertheless, in a large number of specimens examined it has been found that this striking difference holds good no matter what host is attacked. In fact, this progressive sclerotization was the rule in a large number of *L. nigripes* larvae dissected from *Bupalus piniarius* that had been attacked at the laboratory, while in every instance where *L. piniariae* larvae were taken from *Porthetria dispar*, *Nygmia phaeorrhoea*, or any other host the maximum progressive sclerotization was negligible.

In no other respect could a difference in the immature instars of these two species be determined.

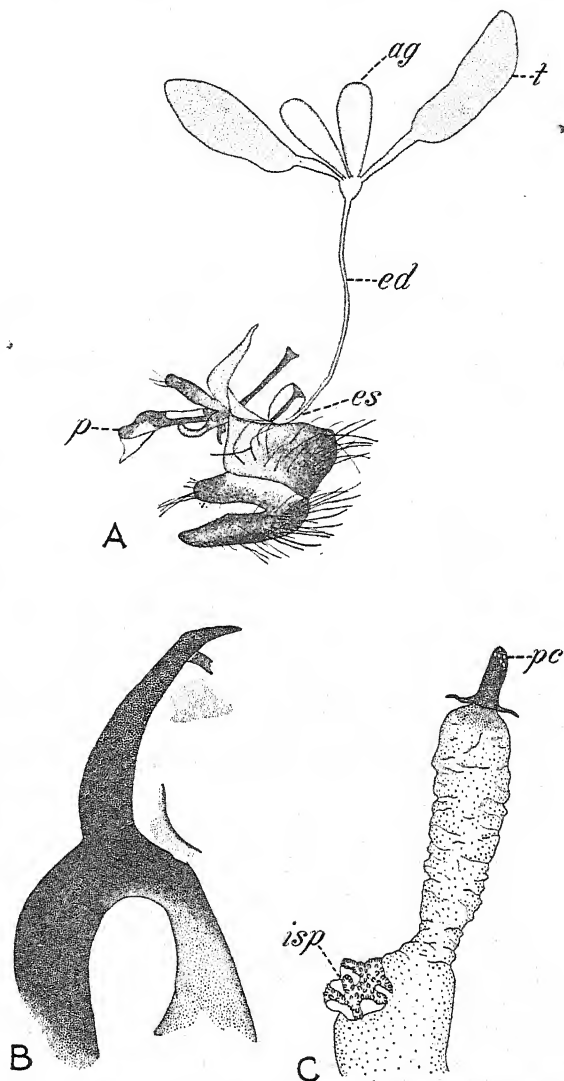


FIGURE 5.—A, Reproductive organs of male *Lydella nigripes*; t, testis; ag, accessory gland; ed, ejaculatory duct; es, ejaculatory sac; p, penis; B, buccopharyngeal armature of first-instar *L. piniariae*; C, pupal respiratory apparatus of *L. nigripes*; pc, prothoracic cornicle; isp, internal spiracle

## BIONOMICS

## SEASONAL HISTORY

## LYDELLA NIGRIPES

The seasonal history of *Lydella nigripes* is clearly indicated by field collections and laboratory rearing. It spends the winter as a larva within the mid-gut of the host pupa or host larva. It completes development, issuing from the host and forming its puparium the latter half of April or early in May. *Porthetria dispar* and *Nygmia phaeorrhoea* must be among the first hosts attacked by the first-generation flies, for puparia are recovered from these species the last week in May and early in June. The first second-generation flies issue from these puparia by June 15 and are ready to attack some other host by the end of June. The writer has not recovered puparia of the next generation from field-collected material, but laboratory records indicate that a third generation of flies would appear in the field about the 1st of August. These flies may deposit maggots which hibernate or which complete development. The first host larva attacked in the laboratory, and in which the parasite hibernated, was *Abrostola tripartita*, larviposition occurring on September 3. Probably most of the maggots deposited by the third-generation flies complete development and produce a fourth generation in the first half of September. The writer has made one field recovery of a puparium formed on August 25 from which a fly issued on September 8. The maggots deposited by the fourth-generation flies live through the winter in the hibernating host, or, if they complete development and issue, produce flies so late in the season that they perish without finding host larvae to attack. The writer has made only one field recovery of overwintering *L. nigripes*. This specimen was reared from a pupa of *A. tripartita* the larva of which was collected on October 3. A fairly large percentage of maggots that were deposited in the laboratory at about this time passed the winter within their hosts. Dissections of hibernating stock showed that most of the parasites hibernated as second-instar larvae, but a few specimens remained in the first instar. All the hosts hibernated as pupae with the exception of *Trichiocampus viminalis*, which spun a cocoon and passed the winter as a larva.

## LYDELLA PINIARIAE

The location of the laboratory made it difficult to conduct field observations on *Lydella piniariae*. The winter is spent as a first-instar larva in the mid-gut of the *Bupalus piniarius* pupa. Development is completed in the spring, and in the material reared by the writer puparium formation was at its maximum about May 27. The first adults from these puparia appeared about June 10, and some females were ready to larviposit by June 20. Under field conditions, however, the females are not ready to larviposit so early as this. Steiner (14) found that maximum issuance of *Bupalus* adults occurred on June 7, whereas maximum issuance of *Lydella* females did not occur until July 2. Eidmann (5) also notes that *L. nigripes*

(= *pinariae*) issued several weeks after *B. piniarius* moths were in flight. *Lydella* females issuing July 2 would be ready to larviposit about July 12, and at this time there are probably young *B. piniarius* in the forest which are large enough to be attacked. At least the writer collected larvae in Austria on July 20 that were easily parasitized in the laboratory, and on the 26th of July 50 field-collected *B. piniarius* larvae were dissected and 2 first-instar *L. pinariae* were found. In *B. piniarius* larvae attacked at the laboratory the maggots did not develop beyond the first instar until the following spring. The species therefore completed but one generation in this single-brooded host. It is believed that this is also the case in nature, for Plotnikov (11) found first-instar larvae in August and September, and although the present writer has dissected a large number of the parasites from *B. piniarius* larvae collected as late as September 17, he has never found any which were developed beyond the first instar.

Possibly *Lydella pinariae* occasionally develops a summer generation in some other species of host. There would be ample time for a generation to develop and still allow the new flies to attack *Bupalus piniarius* larvae in the fall. A large number of host species have been attacked at the laboratory and in two instances a summer generation has been reared. In one of these a larva of *Tortrix dumeana* attacked on August 6 produced a puparium on August 27, and in the other a larva of *Abrostola tripartita* attacked on July 16 produced a puparium on August 19. Eidmann (5) also notes rearing a summer generation of *Lydella* from *Hematarga atomaria* L., but, not having seen Eidmann's specimens, the writer questions whether the flies were *L. nigripes* or *L. pinariae*. However, the large number of host species attacked at the laboratory in which the parasite larvae did not develop beyond the first instar indicate clearly that development of a summer generation is an exception to the rule.

Everything considered, it is probable that *Lydella pinariae* usually has but one generation a year on *Bupalus piniarius*, and only occasionally passes through a summer generation on some other host.

#### LENGTH OF LIFE

##### LYDELLEA NIGRIPES

The flies are sturdy and long-lived under laboratory conditions, where from 5 to 10 are kept in glass-covered wooden boxes 15 by 20 by 10 cm (5.9 by 7.9 by 3.9 inches). As many as 25 may be kept successfully in larger wooden cages (18 by 25 by 20 cm or 7.1 by 9.8 by 7.9 inches) having glass fronts and cloth backs. The flies are fed on lump sugar and a solution of 1 part of honey to 5 parts of water held on sponges. The honey solution is prepared fresh each day. When the flies are not being used they are kept in the dark and, if possible, in a cool place. Table 4 shows the records of a few flies. The flies given only water were held during October and probably survived much longer than they would have if the weather had been warmer.

TABLE 4.—Length of life of *Lydella nigripes* flies held in the laboratory

## FLIES FED SUGAR AND HONEY SOLUTION

Flies used	Flies	Minimum life	Maximum life	Average life
	Number	Days	Days	Days
Mated females used a little for reproduction at laboratory.....	22	13	66	43.4
Males used in mating cages.....	12	12	52	38.9
Unmated females.....	11	50	89	73.7

## FLIES GIVEN NOTHING BUT WATER ON SPONGES

Unmated females.....	5	5	13	9
Males.....	5	5	9	6.5

## LYDELLE PINIARIAE

Although fewer records were kept on length of life for *Lydella piniariae* than for *L. nigripes*, the records show that the former live just as long as the latter under laboratory conditions.

## REPRODUCTIVE SYSTEM

Careful study revealed no difference in the reproductive systems of *Lydella nigripes* and *L. piniariae*.

The external female reproductive organs have been described briefly by Townsend (17). On the venter of the last abdominal segment is a long, heavily sclerotized curved sheath, into the base of which the ovipositor fits and which tapers to a microscopically sharp point. With this organ the female punctures the skin of the caterpillar and introduces the living maggot underneath. Pantel (10) and Townsend (18) have described the same apparatus in detail in *Compsilura concinnata*, a closely allied species.

The internal organs of the unmated female are shown in Figure 6, A. Each ovary is composed of several tubules (fig. 6, B), the number varying with the size of the fly and also often varying slightly in the two ovaries of the same fly. A very small fly may have only 8 tubules and a very large fly 16, whereas an average-sized fly has from 11 to 14. By dissection one can plainly see the outlines of the eggs forming in the tubules at the time the fly issues. Six eggs have repeatedly been counted in a tubule of a freshly issued female and, since this would make from 132 to 168 eggs in an average-sized fly, which is about the number of larvae one finds after development is complete, probably no more eggs are formed. When the fly issues there is one well-developed egg at the base of each ovarian tubule. Those behind it are increasingly small and less developed. As the fly gets older the eggs become larger and more mature, increasing the size of the ovaries.

After the fly is mated the eggs develop rapidly. The mature ones are pushed into the oviducts and pass the spermathecal opening, where they are fertilized, and from there to the uterus, where at first they lie in an even row. The uterus, which is short in the unmated female, gradually lengthens as the eggs descend into it. When full of eggs it consists of four coils within the fly's abdomen. Figure 6, C, shows the reproductive organs of a fly six days after fertilization. Embryonic development progresses rapidly as the eggs descend in

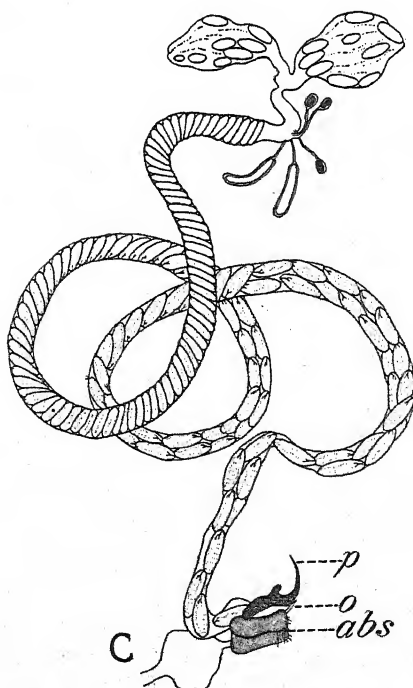
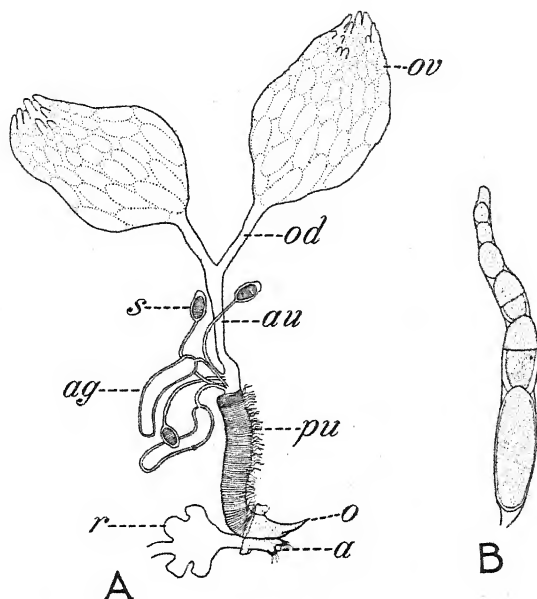


FIGURE f.—Reproductive system of female *Lydella nigripes*: A, Unmated female with piercing organ removed; *ov*, ovary; *od*, oviduct; *au*, anterior uterus; *s*, spermatheca; *ag*, accessory gland; *pu*, posterior uterus; *o*, ovipositor; *r*, rectum; *a*, anus; B, ovariule; C, mated female six days after fertilization, showing piercing organ in position; *p*, piercing organ; *o*, ovipositor; *abs*, sixth abdominal sternite

the uterus. Those at the posterior end are ready to be deposited while those at the anterior end are as yet undeveloped.

In unmated females the pressure of the growing eggs is eventually great enough to force them into the oviducts and then into the uterus.

In a freshly issued fly the abdominal cavity is filled with a large number of fat bodies. These are gradually absorbed by the developing eggs.

The male reproductive organs are shown in Figure 5, A. As the flies become older the testes seem to shorten somewhat.

#### MATING

##### LYDELLA NIGRIPES

Male *Lydella nigripes* do not mate readily until they are a day or two old, but the females mate best when freshly emerged. This peculiarity is well taken care of in nature, for the majority of males invariably issue two or three days before the majority of females. After the females are 2 days old, it is difficult to mate them in the laboratory, and as they get older they resist the males more and more.

In experiments to determine the maximum age at which females might be mated, 6-day-old females were taken in coitu and dissected a few days later. Two of these were apparently fertilized normally. The third had 16 infertile eggs in the lower part of her uterus. These had all been so crowded against the walls by the fertilized eggs behind them that it is believed maggots would have been deposited as usual. Probably ovarian development had forced these infertile eggs past the spermathecal opening before mating took place.

In the laboratory wooden-frame cages measuring 27 by 27 by 65 cm (10.6 by 10.6 by 25.6 inches), covered with cloth, were used for mating. They were kept in the light but not in direct sunlight. Temperatures from 20° to 26° C. (68° to 78.8° F.) are favorable, although at 26° C. the flies are somewhat too active. The morning hours, which are the hours of emergence, are the best for mating.

One female was observed to be mated before her wings were spread. So many males were attracted to this female that it seems possible that such fresh specimens are often fertilized in nature.

The average length of time spent in coitu by 17 pairs was 32 minutes, with a minimum of 14 and a maximum of 48. In one instance a pair disturbed after 29 minutes separated, but 5 minutes later returned and mated for 20 minutes longer.

Female flies never mated more than once, but observation showed that a single male might mate as many as twenty one times and fertilize most of the females. Table 5 shows the record of a male kept at the laboratory. This male was able to fertilize as many as 13 females normally. After that the percentage of infertile eggs rose rapidly, but the eighteenth female, which was mated several days later than the seventeenth, had the normal number of eggs fertilized. Unfortunately the nineteenth female escaped.



TABLE 5.—Mating record of one male *Lydella nigripes* in the laboratory

Date	Number of female fly mated	Time spent in coitu	Eggs found in uterus of female <sup>a</sup>		Date	Number of female fly mated	Time spent in coitu	Eggs found in uterus of female <sup>a</sup>	
			Fertile	Infertile				Fertile	Infertile
		Min-utes	Num-ber	Num-ber				Num-ber	Num-ber
June 21.....	1	-----	-----	-----	June 27.....	12	-----	-----	-----
22.....	2	-----	-----	-----	27.....	13	23	138	2
23.....	3	-----	123	7	27.....	14	25	95	38
24.....	4	-----	126	16	28.....	15	-----	59	10
24.....	5	-----	-----	-----	29.....	16	-----	39	37
25.....	6	-----	84	2	29.....	17	34	68	73
25.....	7	-----	141	1	July 2.....	18	26	106	4
25.....	8	-----	169	1	2.....	19	-----	-----	(c)
26.....	9	20	92	1	3.....	20	-----	None.	All.
26.....	10	23	163	2	7.....	21	-----	None.	All.
26.....	11	22	120	0					

<sup>a</sup> Determined by dissection.<sup>b</sup> As these flies were used some for reproduction, the total number of eggs is incorrect.<sup>c</sup> Fly lost.

## LYDELLE PINIARIAE

The observations noted above for *Lydella nigripes* were found to be the same in *L. piniariae*. The number of times that a male could successfully mate was not determined in such detail, but it was found that one male could fertilize eight females normally and if other females had been available this number would undoubtedly have been increased.

## CROSS MATING

Experimentation on cross-mating *Lydella nigripes* and *L. piniariae* threw considerable light on the difference and also on the close relationship between the two species. It was very difficult to mate the two species, for they showed little interest in each other and in all pairing the females struggled so violently that it is doubtful whether such mating would take place in nature.

The experimentation on this phase of the work is described below:

*Female Lydella nigripes mated with male L. piniariae*.—Five females were mated. They were all apparently normally fertilized. A large number of favored-host larvae were attacked and 23 puparia were formed. Flies issuing from these puparia (first-generation progeny of cross) were kept together and six of the females were mated. These females did not attack favored-host larvae well, but 116 larvae were finally attacked. Reproduction was very poor, and dissection of the females revealed that they had been poorly fertilized. The results of dissecting the six females after they had been used as much as possible in attacking host larvae are shown in Table 6.

TABLE 6.—Relative numbers of fertile and infertile eggs in uterus of six first-generation female progeny of female *Lydella nigripes* and male *L. piniariae*

Female fly No.	Fertile eggs	Infertile eggs	Female fly No.	Fertile eggs	Infertile eggs
1.....	6	92	4.....	10	98
2.....	8	98	5.....	26	130
3.....	5	81	6.....	1	100 plus

Only 5 puparia were secured from the 116 host larvae attacked. Attempts to mate the flies (second-generation progeny of cross) which issued were unsuccessful. It was late in the season and the flies issued over a long period.

*Male Lydella nigripes mated with female L. pinariæ.*—Five females were mated, and all were apparently normally fertilized. A number of host larvae were attacked, but only one puparium was secured. A female fly (first-generation progeny of cross) issued from this puparium.

It is not surprising that so little success was obtained with this cross, for *Lydella pinariæ* was exceptionally difficult to rear in the laboratory. Attempts to rear *Bupalus pinarius* larvae for attack gave poor results, very few pupae being brought through the winter.

*Female fly, first-generation progeny of cross between male Lydella pinariæ and female L. nigripes, mated with male L. nigripes.*—No larviposition was secured from this female. Dissection showed that only eight eggs were fertilized.

*Female fly, first-generation progeny of cross between male Lydella nigripes and female L. pinariæ, mated with male L. nigripes.*—This female practically refused to attack host larvae. Dissection showed 15 fertile and 150 infertile eggs in her uterus.

The results of these experiments indicate a very close relationship between the two species, for mating was secured between them and females of both crosses were fertilized. The experiments indicate, nevertheless, that the two flies are probably different species. Mating was so difficult that it could hardly occur in nature, and there was a very high percentage of sterility among the progeny resulting from such mating.

#### PERIOD BETWEEN MATING AND LARVIPOSITION

Howard and Fiske (7) note that only three or four days are required for the adult female *Lydella nigripes* to reach sexual maturity. The writer was not able to duplicate this record. His experiments showed that the time between the mating of the flies and the first larviposition in both *L. nigripes* and *L. pinariæ* may be as short as six days in very warm weather, but even then the flies did not larviposit eagerly for a few more days. If the weather is cooler, a day or two more is necessary.

#### LARVIPOSITION

A number of different ways of presenting caterpillars to larvipositing flies were tried in the laboratory. The most successful larviposition was secured in wooden boxes measuring 15 by 20 by 10 cm (5.9 by 7.9 by 3.9 inches) and having glass covers. Ten or fifteen larvae were placed in such a box with four or five flies. The box was kept in the light, and as the host larvae were attacked they were removed with a light brush through a hole in the side of the box. Another fairly satisfactory container for this purpose was a wooden box having a glass front and a cloth back. The cloth back was turned toward the light and both caterpillars and flies remained on the cloth fairly well. In both containers it is advisable to keep the larvae in motion.

## LYDELLA NIGRIPES

During the summer months females of *Lydella nigripes* attacked a large number of larvae of different species at the laboratory. Attack was fairly good but decidedly erratic. Hairless larvae were always more readily attacked than hairy ones. It was often very difficult to get *Porthetria dispar* larvae attacked, but there was never the slightest difficulty, under proper conditions of temperature, in having many species of naked larvae parasitized. The optimum temperature for larviposition is between 25° and 30° C. (77° and 86° F.) Flies which refused to larviposit at 21° C. (69.8° F.) did well at 25° C., but the best results ever obtained when using *P. dispar* larvae were at 30° C., when five females deposited in 41 larvae in one and one-half hours.

The fly larviposits by a quick downward and forward movement of the piercer, which breaks the skin of the host larva and allows the larvipositor to enter and deposit the small parasite maggot. A moving caterpillar immediately attracts a fly wishing to larviposit. She quickly runs after it, approaching from the side. Suddenly, with a quick jump forward, she inserts the piercer and larvipositor into the side of the caterpillar and then either passes over it to the other side or flies away. The whole thing is done so quickly that it often seems to be a single motion forward. Often the fly appears to be unsuccessful in her first attempt to larviposit, for she turns around and approaches the larva from the other side. Occasionally as a caterpillar is struck it falls and then the fly falls with it. Sometimes a fly is attracted to a motionless caterpillar and attacks it immediately.

A drop of liquid may exude from the host larva at the point of attack, and the fly often hunts for this liquid and eats it. This is particularly true when larvae of *Bupalus piniarius* and also of *Cadonia punctoria* L., another geometrid, are parasitized. As a rule the fly pays no attention to a larva of *Porthetria dispar* after attacking it.

Species of *Vanessa* larvae, when confined with flies, often strike at them and sometimes smear them with a green regurgitated liquid. This causes the fly to tremble all over and run hither and thither in a crazy manner for several seconds, or even a minute, after which the fly is again normal.

The young maggots are deposited posterior end first. In one or two instances observed the host larva's skin was so thick that it was not completely punctured and the maggot was left partly protruding. In these cases the parasite was not able to gain entrance into the host.

## LYDELLA PINIARIAE

Great difficulty was experienced in getting *Lydella piniariae* females to attack any hairy larvae. All naked larvae, however, were eagerly attacked. Even large geometrid larvae and species of *Smerinthus* larvae, which have skins much too thick for the piercer to penetrate, were eagerly attacked in an attempt to larviposit.

It was particularly desirable to get *Lydella piniariae* to larviposit in *Porthetria dispar*, *Nygmia phaeorrhoea*, and *Vanessa urticae* L. in order to compare its larval development in these hosts with that of *L. nigripes*. Considerable time was therefore spent with these host larvae. Flies of all ages were tried on all larval instars of the hosts. By using larvae with as little hair as possible (in *P. dispar* large third-instar larvae and in *N. phaeorrhoea* fourth-instar larvae) and

by using flies kept continuously in the light, a little larviposition was obtained. The flies would usually run around the cages, over and around the larvae, for hours before they would suddenly attack one.

The *Lydella piniariae* female larviposits in practically the same manner as does *L. nigripes*. She is very apt, though, to jump on a larva, run up and down its back, and larviposit by a movement which shoves the abdomen downward and forward, as if she were sitting down on the larva. The fly shows remarkable ability in staying on a larva, but if the violent motion shakes her off she returns immediately until satisfied.

#### REPRODUCTIVE CAPACITY

The number of larvae deposited by females of both *Lydella nigripes* and *L. piniariae* varies greatly according to the size of the fly. In one very small fly only 65 maggots were produced, while the largest number found was 171 in a very large fly. *L. piniariae* obtained from host larvae collected in the field are a little larger than *L. nigripes* reared from field-collected *P. dispar*.

#### LARVAL DEVELOPMENT

##### LYDELLA NIGRIPES

Embryonic development is complete in *Lydella nigripes*. When a larvipositing female is dissected, the first-instar maggots can be seen actively moving inside their eggshells. At the posterior end of the uterus they are large enough to fill the egg, while at the anterior end they are rather small. It is believed that the act of larviposition usually slips off the delicate eggshell as the larva is forced into the host, for dissections immediately after larviposition almost invariably show larvae outside of the egg membrane. Occasionally, however, an unruptured egg is found.

Immediately after deposition the larva works its way into the cavity of the mid-gut of the host, larvae having been observed in this position five minutes after the host larva was attacked. The parasite larva may attach itself to the wall of the gut by its stigmatic hooks, being thus fastened by its extreme posterior tip with the rest of the body lying free within the gut, or it may enter the gut completely and float freely in the liquid that is found there. About as many larvae have been observed in one position as in the other. The larva may attach itself as it bores through the wall of the gut or later. This point was not determined. Usually, perhaps always, the attachment is at a point where the anal spiracles lie against one of the host's tracheae, and it is believed that the parasite larva may get a supply of air in this way. Nevertheless, the larvae that float freely within the gut surely have no direct contact with the air, and it may be that the tracheal system in this instar is not functional, the peculiarity of attachment being simply the survival of a now nearly extinct habit, at one time useful.

The first-instar larva feeds on the partly digested contents of the host's mid-gut. It usually grows rapidly, but if the food supply is scarce development may proceed quite slowly. The larva measures from 0.90 to 1.28 mm when first deposited. By the time it is 1.40 mm long, usually three or four days later, it invariably shows evidence of the progressive sclerotization of the basal region of the buccopharynx-

geal armature mentioned earlier (p. 970). The first larval instar is completed in the mid-gut of the host; the second instar is also often fully completed and the skin cast either in the position of attachment or while still floating free in the contents of the mid-gut. The well-developed second-instar larva sometimes bores through the gut to get more food. The third instar is spent outside of the gut, with the larva voraciously feeding on the contents of the host's body cavity. This instar completes its development rapidly. Usually the fully developed *Lydella nigripes* larva issues from the host larva. Occasionally the host pupates before development of the parasite larva is complete, but then it issues readily from the pupa. Issuance is made at any convenient point. The larva cuts a hole with its mouth hooks, emerging head first. A few cases have been noted where the parasite, instead of issuing, forms the puparium within the dead host's skin or pupal shell.

More than one *Lydella nigripes* can develop in the same host larva. In one instance three developed in a single *Porthetria dispar* caterpillar, and once seven normal-sized puparia were obtained from a single larva of *Polia brassicae*.

The time spent in each larval instar varies considerably according to the temperature, size of host, etc. A few specimens were dissected each day to determine growth. The quickest development noted was in *Vanessa urticae* larvae attacked in the laboratory on July 5. Four days were spent in the first instar, 3 in the second, and 3 in the third. Other records showed that 50 days were required for larval development in retarded *Porthetria dispar* larvae that were attacked on August 28.

After leaving the host, the third-instar larva burrows into the ground a short distance to form its puparium. In the laboratory the puparium is readily formed without earth or other covering material. It is believed, however, that in nature the larvae always enter the ground, for when given an opportunity in laboratory experiments they always do so. Ten larvae placed on rather firmly packed soil in the laboratory burrowed down to an average depth of 3.1 cm (1.2 inches), with a maximum of 4.0 cm (1.6 inches) and a minimum of 1.0 cm (0.4 inch). Outdoors six larvae burrowed to an average depth of 1.2 cm (0.5 inch), with a minimum of 0.6 cm (0.2 inch) and a maximum of 1.8 cm (0.7 inch). Before forming their puparia, all the larvae under observation turned around so that they lay with their heads pointed upward.

#### LYDELLA PINIARIAE

Larval development in *Lydella piniariae* is slightly different from that in *L. nigripes*. Hatching and entrance into the mid-gut of the host are the same, but in *L. piniariae* the larva always attaches itself to the intestinal wall and it never shows progressive sclerotization of the basal lobes of the buccopharyngeal armature. The first-instar larva of *L. piniariae* develops so slowly that it passes the winter in this stage within the host pupa. Possibly the reason the larva always attaches itself to the wall of the gut is that it spends such a long time in this position that provision must be made for an air supply.

Development in the spring seems to follow no definite rule. Sometimes the parasite cuts two openings in the host's gut, one near the

parasite's anal spiracles and the other near its mouth, and holds a mass of the host's body material in a rather firm lump at each of these points. These masses are full of tracheal tubes, and at the posterior end of the parasite's body many of them are held by the stigmatic hooks. The larva feeds on the mass of material held anteriorly. At other times the gut is ruptured at only one point, and one larva was observed which had reached the second instar without puncturing the gut at all. In general, though, the gut is punctured at the end of the first or at the beginning of the second instar, and as the larva feeds it surrounds itself with a brownish, filmlike protective covering of host remains. The second-instar larva is also attached to one of the host's tracheae. The third-instar larva is inclosed in a protective covering or sac as before, but it has never been observed attached to a trachea. There is so much variation in dissected material that it was impossible to determine accurately the duration of the second and third larval instars, but development is probably rather slow in nature, for puparia do not appear from collected pupae until the latter part of May or early in June.

It is not uncommon to find more than one *Lydella piniariae* maggot in a *Bupalus piniarius* larva in the fall, but the writer never reared more than one parasite larva from a host pupa in the spring, and dissections of pupae never showed more than one present, except in one instance, where there were two third-instar larvae, one of which was dead. Apparently a larva of *L. piniariae* is always able in some way to eliminate competitors of the same species. Marks have been noted on dead larvae that were undoubtedly made by the mouth hooks of another.

When feeding is completed, the larva leaves the host and burrows into the ground as was noted for *L. nigripes*.

#### PERIOD SPENT IN THE PUPARIUM

##### LYDELLA NIGRIPES

Data on the period of time between the formation of the puparium and the issuance of the flies were obtained from laboratory-reared material. As the puparia were formed, they were isolated and then examined daily for issuance of flies. Table 7 shows the results obtained.

TABLE 7.—Time spent by *Lydella nigripes* in the puparium

Time of formation of puparium	Puparia producing male adults	Puparia producing female adults	Average time spent in puparium	
			Males	Females
	Number	Number	Days	Days
Apr. 25 to May 1.....	12	10	17.5	17.9
May 31 to June 5.....	14	10	12.3	12.6
July 1 to 15.....	1	3	13.0	12.3
July 16 to 31.....	42	55	11.9	12.8
Aug. 1 to 15.....		1		14.0
Aug. 16 to 31.....	2	8	12.0	14.7
Sept. 1 to 15.....	20	28	12.2	14.0
Sept. 16 to 30.....	56	89	15.7	16.8
Oct. 1 to 15.....	7	4	19.0	25.0
Oct. 16 to 31.....	3	2	26.0	31.0

The time spent in the puparium varies greatly with the temperature. Male adults issue in from 12 to 14 days in June, July, and August, but in October as many as 26 days may be required. Females require a day or so longer. This peculiarity is common among the Tachinidae, and undoubtedly is an important factor in mating, for the best mating is secured between freshly emerged females and males from 2 to several days old. When a number of *Lydella nigripes* pupae are secured from simultaneously attacked host larvae, the first-formed pupae always produce a large percentage of males and those formed later a large percentage of females.

Toward the end of the rearing season the majority of flies that issued were females. One case was noted where 17 larvae of *Polia brassicae* attacked between August 25 and September 4 produced 33 puparia between September 6 and 19, and from these puparia 12 male and 21 female *Lydella nigripes* flies issued. It would be almost impossible to check such results in nature, but the phenomenon might be a natural one, for we know that males mate several times, and there is therefore less need for equal numbers of each sex toward the end of the summer.

#### LYDELLE PINIARIAE

The length of time *Lydella piniariae* spends in the puparium was also observed under laboratory conditions. The puparia were part of a large number secured from overwintering *Bupalus piniarius* pupae. The time was found to be about the same as for *L. nigripes*. The fact that the majority of male larvae complete development earlier in the season than the females is clearly brought out in Table 8.

TABLE 8.—Issuance of *Lydella piniariae* flies from two lots of puparia

Time after formation of puparia (days)	From a lot of 540 puparia formed May 24, 1929		Time after formation of puparia (days)	From a lot of 211 puparia formed June 4, 1929	
	Adult males issuing	Adult females issuing		Adult males issuing	Adult females issuing
	Number	Number		Number	Number
14.....	110		14.....	14	
15.....	120		15.....	22	18
16.....	115	10	16.....	7	70
17.....	33	49	17.....		46
18.....	3	20	18.....		2
19.....		2	19.....		3

#### LABORATORY REARING

##### LYDELLE NIGRIPES

*Lydella nigripes* was reared at the laboratory on retarded *Porthetria dispar* in order to get material for study. A large number of other hosts were also exposed to attack, in an attempt to get data on the different host species in which it could develop. The species in which *L. nigripes* was reared are listed earlier in this paper (page 965). Development in *P. dispar* larvae was rapid in most cases, but there was considerable variation in the time spent as a larva. In *P. dispar* stock attacked the latter part of August, many of the parasite larvae developed very slowly and a few of them did not develop

beyond the first instar. It is believed that the retarded host larvae did not give sufficient nourishment to the parasites, for, with the exception of *Bupalus piniarius*, development was rapid in other hosts attacked at this time. This species can hardly be compared with the others, however, for most of the larvae were so small when attacked that development was retarded. Furthermore, in practically every case *L. nigripes* completed development in *B. piniarius*. *L. piniariae*, on the other hand, never developed beyond the first instar in this host, during the summer. *L. nigripes* larvae always showed the progressive sclerotization of the basal lobes of the pharyngeal armature after they had developed in *B. piniarius* larvae for a few days, but this never occurred in *piniariae* larvae.

The smallest *Porthetria dispar* larvae successfully attacked at the laboratory were large second-instar caterpillars.

#### LYDELLA PINIARIAE

Results from attempts to rear *Lydella piniariae* larvae at the laboratory were very different. A fairly large number of hosts were attacked and they include many species in which *L. nigripes* completed a summer generation. Nevertheless, the only evidence obtained which indicated that a summer generation of *L. piniariae* might develop, was one puparium formed on August 27 from a pupa of *Tortrix dumeana*, the larva of which was attacked at the laboratory on August 6, and one puparium formed August 19 from a larva of *Abrostola tripartita* attacked July 16. A list of larvae attacked by *Lydella piniariae* follows. Those starred (\*) are species in which *L. nigripes* develops a summer generation.

- |                                      |  |
|--------------------------------------|--|
| *2 <i>Abrostola tripartita</i> Hufn. | *53 <i>Nygmia phaeorrhoea</i> Donovan. |
| 2 <i>Agrotis pronuba</i> L.          | 1 <i>Pieris brassicae</i> L.           |
| 1 <i>Amphidasia betularia</i> L.     | 6 <i>Pieris rapae</i> L.               |
| *25 <i>Bupalus piniarius</i> L.      | *2 <i>Plusia gamma</i> L.              |
| *7 <i>Calophasia casta</i> Bkhn.     | *225 <i>Porthetria dispar</i> L.       |
| 43 <i>Cadonia punctoria</i> L.       | 2 <i>Smerinthus quercus</i> Schiff.    |
| 2 <i>Diphthera alpium</i> Osbeck.    | *4 <i>Synopsis sociaria</i> Hbn.       |
| 1 <i>Drymonia querna</i> Fab.        | 8 <i>Tortrix dumeana</i> Freyer.       |
| *38 <i>Polia brassicae</i> L.        | 81 <i>Vanessa urticae</i> L.           |
| 9 <i>Melitaea</i> sp.                | 3 <i>Vanessa io</i> L.                 |

In general the larvae attacked lived a long time. Most of them lived long enough for the parasites to develop, but dissection of the stock showed that the parasite larvae did not develop beyond the first instar. Two first-instar larvae were found in *Porthetria dispar* caterpillars 18 days after larviposition, two first-instar larvae were found in *Nygmia phaeorrhoea* larvae 17 and 24 days after attack, and one first-instar larva was found in *Vanessa urticae* 17 days after larviposition. These facts indicate that as a rule *Lydella piniariae* does not develop beyond the first instar in the summer, but is essentially a single-brooded species. The two exceptions noted indicate that the species may occasionally have a summer generation.

#### COMPARISON OF LYDELLA NIGRIPES REARED FROM PORTHETRIA DISPAR AND FROM NYGMIA PHAEORRHOEA

Puparia of *Lydella nigripes* were obtained the last part of May and the early part of June, 1930, from field-collected larvae of *Porthetria dispar* and *Nygmia phaeorrhoea*. In order to avoid any possible con-



fusion between flies reared from these two hosts, the material originating from *P. dispar* and that originating from *N. phaeorrhoea* were kept separate for the entire season. Material was cross-mated in both directions with no difficulty, and the progeny resulting from both crosses were reared for three generations. No difference was noted in the larvae or adults reared from the two hosts or from cross-mated stock.

#### COMPARISON OF *LYDELLA NIGRIPES* WITH *COMPSILURA* *CONCINNATA*

Although the adults of *Lydella nigripes* and *Compsilura concinnata* show morphological differences, the similarity in their biology and immature stages has been noted in several instances. Townsend (17) in 1908 and Pantel (10) in 1910 first called attention to this, and Howard and Fiske (7, p. 296) in 1911 noted that *Lydella* has a more restricted host relationship than *Compsilura* and "is exceedingly rare as a parasite of the gipsy-moth caterpillars," but "in every other respect except host relationship, the habits of the two are identical, and so far as known the earlier stages are absolutely indistinguishable."

So far as host relationships are concerned, it is true that, in spite of the large number of hosts which *Lydella* attacks, the number which *Compsilura* is known to parasitize is far greater. *Lydella nigripes*, however, may at times be of some importance as a *dispar* parasite.

It seemed worth while to try again to detect differences between the immature stages of the two parasites. No way of separating the first-instar larvae was found, but those of the second and third instars, as well as the puparia, are quite distinct. The anterior spiracles of second-instar *Lydella nigripes* larvae almost invariably have 3 papillae, rarely 2, and very seldom 4. In *Compsilura*, on the other hand, there are almost always 6 or 7 papillae. In only one instance has an exception, where there were 5, been noted. The third-instar larvae may also be separated by the number of papillae of the anterior spiracles. In *Lydella* there are 3 or 4, in *Compsilura* 5 to 7. The species may further be distinguished in this instar by the arrangement of the cuticular spines surrounding the posterior spiracles. In *Lydella* there are 2 small patches of spines which coalesce at their bases, situated just anterior to the posterior spiracles, and 1 or 2 similar patches placed close together posterior to the spiracles. In *Compsilura* one finds rather heavy spines, but they do not coalesce at their bases into patches. The puparia are distinct. In *Lydella nigripes* the posterior end is somewhat pointed, the spiracular plates are nearly contiguous, and the area just below them is distinctly raised. The posterior end of the *Compsilura concinnata* puparium is rounded, the spiracular plates well separated, and the area below them practically flush.

These differences, although important aids in the determination of the immature forms, do not alter the fact that the two species are biologically very closely related. The habits of the flies—larviposition, larval development, and seasonal history—are all so nearly identical that one is amazed that *Compsilura* became so easily and widely established in North America, while, so far as known, *Lydella* with the same opportunities could not become acclimatized. The reasons for this remain a mystery, but one or two points noted in

studying the flies may be interesting in this connection. In the first place there is a very marked difference in the way the two species larviposit. *Compsilura concinnata* eagerly attacks almost any species of host larva whether it is hairy or not. The flies attack with such vigor that they easily puncture the skin with their sharp piercers and deposit their maggots. Flies of *Lydella nigripes*, on the other hand, do not attack hairy larvae at all eagerly; in fact, the attack is often not aggressive enough to push the piercer through the host larva's skin. They seem quickly discouraged or tired and soon can not be induced to attempt larviposition at all. Another, perhaps even more important, difference is the fact that *Compsilura* larvae usually survive better than those of *Lydella*. General laboratory rearing of these parasites in larvae of *Porthetria dispar* and *Nygmia phaeorrhoea* showed that invariably a high percentage of the larvae attacked by *Compsilura* gave puparia, while even the larvae which were surely attacked by *Lydella* (as evidenced when a drop of liquid exuded at the point of attack) produced a low percentage of puparia. The reasons for this could not be determined. Dissections almost always showed healthy larvae, although occasionally none could be found. A third consideration which might help explain why *Compsilura concinnata* is so successful in America whereas *Lydella nigripes* has not become established is that of competition. The two species must be severe competitors in nature. In Europe, it is true, they exist side by side, although apparently neither ever reaches a very high degree of effectiveness as a parasite.

In North America *Compsilura concinnata* is very successful. It has perhaps been able to fill its place in the American fauna so well that *Lydella nigripes*, which must compete for nearly the same place, can not establish itself.

Laboratory experiments on the competition between *Compsilura concinnata* and *Lydella nigripes*, as indicated in the following section, seem to show that *Compsilura concinnata* is the stronger of the two, but in a number of instances both parasites developed in the same host larva.

#### COMPETITION WITH OTHER PARASITES

##### LYDELLA NIGRIPES

No data were secured in the field regarding the competition between *Lydella nigripes* and other parasites in *Porthetria dispar* or *Nygmia phaeorrhoea*. There might be rather severe competition between *Lydella nigripes*, *Compsilura concinnata*, *Carcelia separata* Rond., *Sturmia inconspicua* Meig., and *Hyposoter* spp. in *Porthetria dispar*, for they all attack and issue from the host about the same time. In *Nygmia phaeorrhoea*, *Lydella nigripes* has *Compsilura concinnata*, *Sturmia nidicola* Townsend, *Pales pavidus* Meig., and *Zenillia larifrons* Villeneuve as severe competitors.

In the laboratory an experiment in competition was carried on between *Lydella nigripes* and *Compsilura concinnata* in *Porthetria dispar* larvae. Third and fourth instar host larvae were used. Such conditions may never occur in the field, but the results are given in Table 9 as having at least an academic interest.

TABLE 9.—*Competition between Lydella nigripes and Compsilura concinnata in third and fourth instar Porthetria dispar larvae attacked at the laboratory*

Parasite	Host larvae attacked	Host larvae from which puparia of the parasite were obtained			
		<i>Comp-silura</i>	<i>Lydella</i>	<i>Lydella</i> and <i>Comp-silura</i>	Total
<i>Lydella</i> (check).....	50	—	24	—	24
<i>Compsilura</i> (check).....	50	20	—	—	20
<i>Lydella</i> and <i>Compsilura</i> simultaneously.....	65	18	11	7	36
<i>Lydella</i> and 2 days later <i>Compsilura</i> .....	63	12	7	1	20
<i>Lydella</i> and 4 days later <i>Compsilura</i> .....	63	6	10	1	17
<i>Compsilura</i> and 2 days later <i>Lydella</i> .....	63	17	3	—	20
<i>Compsilura</i> and 4 days later <i>Lydella</i> .....	63	40	5	—	45

## LYDELLA PINIARIAE

No data were secured from the field on competition between *Lydella piniariae* and other parasites in *Bupalus piniarius*, but the competition must be very keen, for a large number of hymenopterous parasites are reared from this host.

## FACTORS LIMITING THE EFFECTIVENESS OF THE TWO SPECIES AS PARASITES

## LYDELLA NIGRIPES

It is rather surprising that *Lydella nigripes*, which is usually present in some numbers in infestations of *Porthetria dispar*, should be of no greater importance as a parasite of that host. There does not seem to be any reason why the species should not be at least potentially as effective as *Compsilura concinnata*. Experimentation seems to indicate, however, a few of the reasons why it does not increase more. Perhaps the most important is that *Lydella* seems to prefer hairless larvae for attack. If this is as true in the field as it is in the laboratory, it can be understood why *dispar* might be avoided when there was an abundance of preferred host material in the field. The fact that the species is very polyphagous also cuts down its effectiveness as a *dispar* parasite, since it is not obliged to confine its efforts to this host. Another fact that may limit the species is that a large percentage of the maggots deposited in the late fall complete development at such a late date that the issuing flies do not find larvae to attack, and consequently perish without reproducing. In one instance 21 larvae of *Abrostola tripartita* were attacked at the laboratory on October 7. Nine puparia were formed on November 6, from which flies issued about December 10, much too late for them to find larvae to attack. Only four puparia were recovered from the hibernating pupae in the spring. If the same tendency is shown in the field there must be a very high mortality.

*Lydella nigripes* has generally shown itself a more effective parasite of *Nygmia phaorrhoea* than of *Porthetria dispar*, but it has the same limitations on this host also.

During this work there was no opportunity to collect *Lydella nigripes* puparia in the field in order to ascertain whether or not the species is attacked by secondary parasites. It spends such a short

time in the immature stages that it is probably not seriously parasitized, but laboratory notes for 1906, 1907, and 1908 indicate that a few *Monodontomerus aereus* Walker were reared from the puparia.

#### LYDELLA PINIARIAE

*Lydella piniariae* is much more effective than *L. nigripes*. The percentage of parasitization is often very high, and in every case under the writer's observation it showed a marked increase during the second and third years of the infestation. All the limiting factors that were noted for *L. nigripes* are inapplicable to *L. piniariae*. It eagerly attacks *Bupalus* larvae, goes into hibernation early in the summer, and probably confines itself almost exclusively to the one host. As a matter of fact, there does not seem to be any reason why the parasite should not go on increasing at the expense of the host at such a rate that it would soon eliminate it, and it is believed that this would actually take place if the combined efforts of the parasites attacking *B. piniarius* did not check an infestation before *L. piniariae* reached its full effectiveness. Of course, there are some rather obvious limiting factors, and one of them may be serious enough to hamper greatly the parasite's effectiveness at times. This is a hyperparasite, *Mesochorus politus* Grav., which attacks the parasite while it is within its host. More than 57,000 *L. piniariae* pupae were reared in 1929, and this secondary parasite was not encountered, but in 1930 out of a lot of 600 puparia no less than 299 adults of *M. politus* issued. Sitowski (13) notes that he found this hyperparasite destroying 10 per cent of the *L. piniariae* pupae. A limiting factor of less importance is the fact that only one *Lydella* larva can develop in a single *B. piniarius* larva. This must result in a rather heavy mortality among the parasites for, of course, one host may be attacked several times. There must also be severe competition between *L. piniariae* and the large number of parasitic species which inhabit *B. piniarius*. The parasite must also suffer greatly when the host pupae fail to live through the winter owing to climatic conditions, fungus, etc.

#### SUMMARY AND CONCLUSIONS

*Lydella nigripes* Fallén is a polyphagous tachinid parasite in Europe. Because it is of some importance in controlling the gipsy moth (*Porthetria dispar* L.) and the brown-tail moth (*Nygmia phaeorrhoea* Don.), it seemed desirable to try to establish it in the United States. During the summer it completes three and often four generations in different host species. It hibernates as a second-instar larva within the host pupa or larva.

*Lydella piniariae* Hartig is an important European parasite of the pine geometrid (*Bupalus piniarius* L.). The host larvae are attacked during the summer and the parasite develops very slowly, hibernating as a first-instar larva in the mid-gut of the host pupa. As a rule the parasite probably completes only one generation each year in this single-brooded host, but a summer generation may occasionally develop in some other host species.

The two species have previously been considered identical, but soon after this study was begun it became apparent that there was a difference in the biology of flies reared from *Bupalus piniarius* and those reared from *Porthetria dispar* and *Nygmia phaeorrhoea*. It was

possible to separate the adults morphologically. The immature stages have been studied and found to be almost identical. The only differences noted were in the first-instar larvae. The buccopharyngeal armature of *Lydella nigripes* shows progressive sclerotization of the basal lobes as the larva becomes fully developed, whereas this does not occur in *L. piniariae*, and the tip of the buccopharyngeal armature in first-instar *L. nigripes* is more sharply angled than in *L. piniariae*.

The adults of *Lydella nigripes* and *L. piniariae* show little difference in behavior. Both are long-lived. They mate readily in the laboratory and the males are capable of mating many times. Experimentation in cross-mating the two species indicated that they probably would not mate in nature. Cross-mated females were fertilized, but the progeny were nearly sterile.

The females of *Lydella nigripes* and *L. piniariae* have a sharp, stout piercer attached ventrally on the last body segment. The base of the ovipositor fits into this instrument and the fly uses it to puncture the host larva's skin. As the skin is punctured a living maggot is deposited inside the host. From 125 to 150 maggots are deposited by average-sized females of both species.

*Lydella piniariae* practically refuses to attack hairy larvae, whereas *L. nigripes*, although preferring naked larvae, attacks many species of hairy caterpillars.

There are some differences in the larval development of the two species. The first-instar larva of *Lydella piniariae* is always attached to the wall of the host's mid-gut, the second-instar larva is apparently always attached to a host trachea, and the third-instar larva is found in a protective sac or covering formed from the host remains. About as many of the first-instar larvae of *L. nigripes* float free in the liquid contents of the mid-gut as attach themselves to a trachea, many of the second-instar larvae float free in the same position, although some attach themselves to a trachea, and the third-instar larvae may or may not form a protective covering or sac from the host's remains. The mature larvae of both species leave the host and burrow a short distance into the ground to form puparia. The males spend about 11, the females 12, days as pupae.

*Lydella nigripes* regularly completed a summer generation in larvae of *Porthetria dispar*, *Nygmia phaeorrhoea*, *Bupalus piniarius*, and many other host species attacked at the laboratory. *L. piniariae*, on the other hand, rarely completed a summer generation. In two isolated instances puparia were recovered, but deposited maggots never developed beyond the first instar in *B. piniarius*, *P. dispar*, *N. phaeorrhoea*, or a number of other host species in which *L. nigripes* developed rapidly.

Although there are some marked differences in the life histories and habits of *Lydella nigripes* and *L. piniariae*, there are similarities in almost every phase of their life histories which show that a very close biological relationship exists between them. The obvious explanation is that they present an example of species evolution, which has not progressed far enough to bring about marked morphological changes. Since *L. piniariae* may be considered as simpler than *L. nigripes*, it is probably the older.

A brief comparison was made between *Lydella nigripes* and *Comptosia concinnata*, a parasite of *Porthetria dispar* and *Nygmia phaeor-*

*rhoea* which has been very successfully established in the United States. The life histories, habits, and immature stages of the two parasites are remarkably similar. The first-instar larvae could not be separated, although slight differences were noted in the later instars. The two species undoubtedly compete severely in nature. In the laboratory *C. concinnata* seems to be somewhat stronger, but larvae of both species often developed in the same host larva. *C. concinnata*, nevertheless, is much more aggressive. It eagerly attacks hairy caterpillars as well as naked larvae. Perhaps this is why *C. concinnata* has been able to establish itself so well in the American fauna while *L. nigripes* has been unsuccessful.

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# POROSITY AND WATER ABSORPTION OF FOREST SOILS<sup>1</sup>

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## INTRODUCTION

The manner in which water in the form of rainfall or run-off acts, upon the surface soil, and the capacity of the soil to absorb water, are well recognized as two interrelated factors that link together all problems of soil erosion, flood control, and water conservation. Two other factors that, less obviously, are linked to these through their effect on surface soils are land abandonment on the one hand and forestry on the other.

In the Central States, where the studies here detailed were pursued, land abandonment has reached disturbing proportions, and the end is not yet. The 1925 census records 6,500,000 acres of idle and fallow

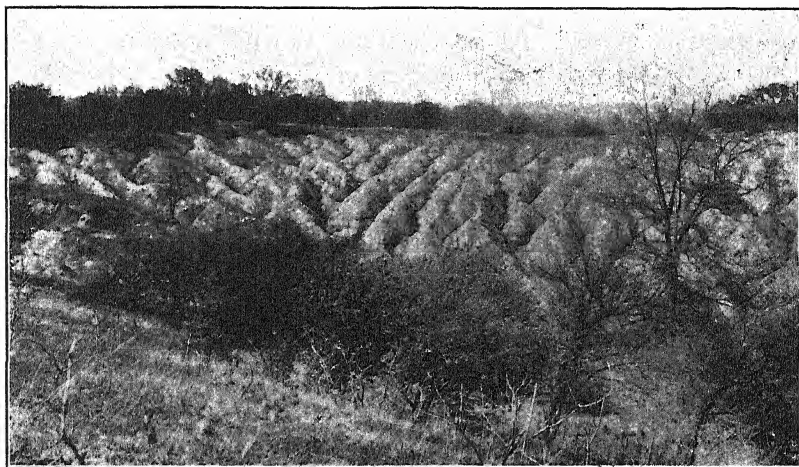


FIGURE 1.—A field that has become a waste of ever-growing ditches

land in these States; and a recent survey of a typical southern Ohio county (6)<sup>3</sup> reveals 50 per cent of the improved land abandoned since 1900.

With abandonment of poor land came erosion. Indeed, soil erosion started on the steeper slopes and hills as soon as the forest cover was cleared away, but reached much greater proportions as unproductive fields were abandoned to the elements. The loss from erosion under faulty methods of managing hill lands is doubtless more wasteful and destructive than the loss of plant food through cropping. The surface soil itself is quickly washed away by heavy rains and gullies form and grow until what was once a field becomes a waste of ever-growing ditches. (Fig. 1.)

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<sup>2</sup> Maintained in cooperation with Ohio State University and Ohio Agricultural Experiment Station.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 1014.

The influence of forests and the practice of forestry are all in the other direction of holding and building up surface soils and, perhaps more important, of lightening soil structure and so increasing porosity. Under a cover of forest trees, erosion is not a problem where the forest is not repeatedly burned or severely overgrazed. The tree roots and protective cover of leaf litter keep the soil from washing. The same conditions which prevent erosion favor water conservation. Forest soils protected by tree cover and leaf litter retard the effect of surface run-off and thereby conserve the moisture which is precipitated in the form of rain and snow. This water is then held in storage as a feeder of wells and springs and, slowly released, stabilizes stream flow and tends to check excessive floods.

Thus it is that, whereas problems of floods, erosion, and natural reservoirs are in the Central States largely problems of soil management, one solution, and often the only solution, of such problems lies in putting idle land to work through the growing of protective and productive crops of forest trees.

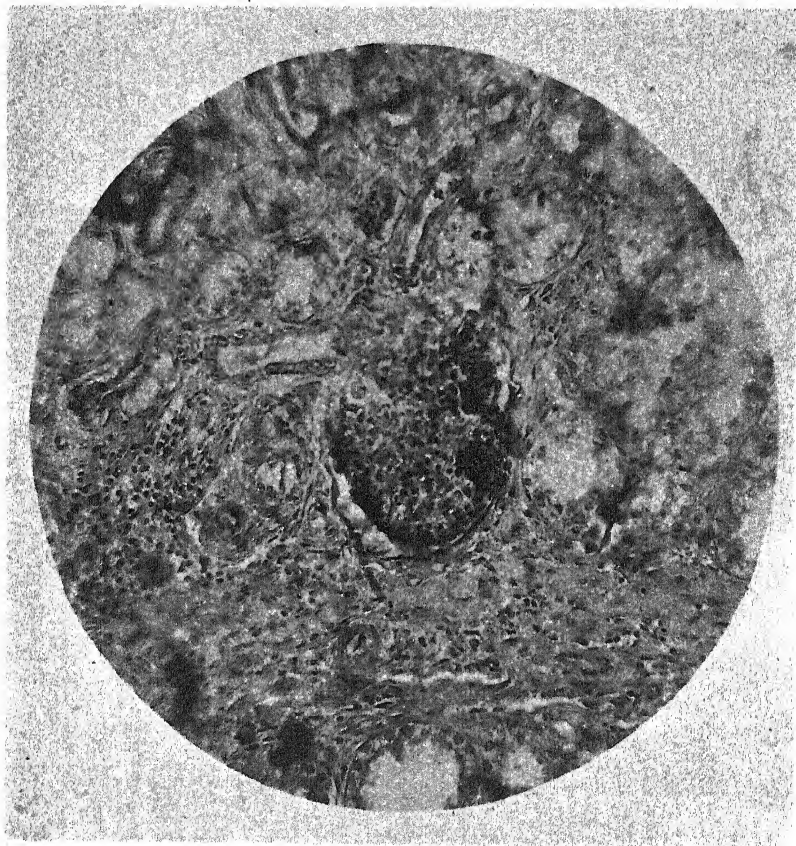
While the influence of forests and forest litter in the control of the aforementioned problems is recognized, and reforestation of waste land is proposed as one of the important solutions, yet the changes in soil structure, fertility, and moisture content undergone in the transition from forest to field conditions have not been understood thoroughly enough for foresters to appreciate the difficulties in starting new tree growth on waste land.

In order to cope with these conditions, it is necessary for the forester to take advantage of certain soil relationships and to apply some basic facts regarding the soil to the problems of forest management. One approach is through a study of the functions of forest litter.

The literature pertaining to litter, and especially to its effect upon soil porosity and water absorption, is meager. Eiffert (5, *p.* 1266-1269), assuming 0.12 inch of rainfall to be held by the average forest litter, pointed out the insignificance of this amount in its effect on the Mississippi floods. Lowdermilk (3) recognized that the absorbing capacity of forest soil as a function of its porosity is to some extent independent of litter cover. McCarthy (7, *p.* 102) emphasized the importance of forest soil structure in influencing water-holding capacity. Munns (4) estimated the maximum excess of water held in forest over field soil to be about 1 inch, but stated that with good forest practice this amount might be increased. Korstian (2) recognized the difference between litter-covered and bare soils and found the penetrability of litter-covered soil to be 175 per cent greater than that of bare soil. Warington (8, *p.* 47), in commenting on two Rothamsted soils, stated that the soil densities increased to a depth of 36 inches and were fairly constant below that level. Harmer (1), when reporting on the densities of six field soils and six open prairie soils, found them about equal in compaction. He stated that the density increased with depth rather gradually in prairie soils, but more rapidly in forest soils.

To arrive at a better understanding of the relation of soil to the problems of forestry and to determine the influence of forest cover and forest litter, a series of studies has been undertaken, out of which the study of soil porosity and capacity for water absorption was chosen for immediate investigation because of its important bearing on the problems of erosion, flood control, and water conservation, as





Photomicrograph of a section of a kidney from a pig fed 2 per cent rock phosphate, showing characteristic degeneration and presence of fibrous tissue.



well as its relation to the successful establishment and maintenance of forest plantations on the farm and on waste land.

The work in this investigation covered both soil porosity and water-absorbing capacity. Because of contrasting conditions of forest and field soils, a comparison of these characteristics was made in four distinct phases, as follows: (1) Old-growth forest soil with open-field soil; (2) second-growth forest soil with open-field soil; (3) grazed with ungrazed second-growth forest soil; (4) forest-plantation soil with open-field soil.

#### SOIL POROSITY OF OLD-GROWTH FOREST AND OPEN FIELD

From among the very few forest stands in the Central States undisturbed by cutting, grazing, or recent fire, 22 areas were selected



FIGURE 2.—Characteristic old-growth virgin soil

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that were near enough to a natural condition to be suitable for the purposes desired. (Fig. 2.) These sites, in Ohio, Indiana, Michigan, and Illinois, were selected on the basis of no grazing, no cutting for at least 50 years except for the removal of an occasional tree, and no recent fire damage apparent. The absence of grazing was determined by the uniform distribution of age classes and by the available history of the woods. So far as could be determined from observation and checking with soil-survey data, the soils compared were of the same original type.

Twenty soil tests for apparent specific gravity, or air-dry weight divided by a constant volume, were taken on each area—10 in the forest and 10 in adjacent fields. This number varied in a few instances, but forest and field samples were paired on each site. Each

field site was selected as near the forest as possible as a precaution against variation in soil type between the two. The topography was observed and the samples taken as near the same elevation as possible. All surface litter and duff were removed from the forest soil in order that the comparisons might be made purely on the basis of mineral soil. The cultivated soil did not, of course, have the surface organic horizon. In addition to the 10 specific-gravity samples on each site, a 500-g sample was taken for moisture and real specific gravity determination. This latter sample was weighed immediately, placed in a pint cardboard box, and taken to the laboratory.

The apparent specific-gravity tests were made with the usual specific-gravity tube, a hollow iron cylinder  $2\frac{1}{2}$  inches in diameter and  $12\frac{1}{4}$  inches long. One end was beveled on the outside to make a sharp cutting edge. This bevel, being on the outside, did not change the uniform inside diameter of the tube; hence, significant compression of soil did not result when the tube was driven in. With an iron plug turned from high-grade machine steel as a driving head and an 8-pound steel sledge, the tube was driven into the soil until only 3 inches projected above the surface, as determined by a groove machined around the tube. The core of soil  $2\frac{1}{2}$  inches in diameter and  $9\frac{1}{4}$  inches long (6.35 by 23.5 cm with a volume of 744 cm<sup>3</sup>) was removed from the tube and weighed.

TABLE 1.—Average weights of constant soil volumes (744 cm<sup>3</sup>) to a depth of 9.25 inches compared in various old-growth forest areas and adjacent open fields <sup>a</sup>

Forest soil location	Forest soil	Open area	
		Description	Soil sample
	<i>Grams</i>		<i>Grams</i>
Keffer Woods, Mifflin, Ohio.....	<sup>b</sup> 945.6	Wheat stubble.....	<sup>b</sup> 1,087.1
State Sanatorium Woods, Mount Vernon, Ohio.....	912.8		1,061.2
Myers Woods, Oldenburg, Ind.....	956.2		1,053.0
Berkey Woods, Warsaw, Ind.....	1,155.7		1,244.4
Rosebrook Woods, Warsaw, Ind.....	1,010.4		1,078.2
Pines Park Woods, Dixon, Ill.....	970.2		1,114.4
Bent Tree Woods, Elizabeth, Ill.....	859.0		999.1
Foley's Woods, Paris, Ill.....	857.4		1,133.1
Rhymer Woods, Dongola, Ill.....	857.1		1,082.7
Turkey Run, No. 1, Marshall, Ind.....	<sup>c</sup> 879.1	Clover field.....	1,058.0
Turkey Run, No. 2, Marshall, Ind.....	<sup>d</sup> 900.5		1,058.0
Dillon Woods, Porter, Ind.....	928.2		1,013.2
Spring Mill Park, Mitchell, Ind.....	905.7	Cornfield.....	1,019.7
Lewis Woods, Williamsburg, Ind.....	860.5		1,079.5
Purdue University Woods, Farmland, Ind.....	916.1		1,042.0
Brownfield Woods, Urbana, Ill.....	914.3		1,134.4
Fricke Woods, Jonesboro, Ill.....	<sup>e</sup> 996.7	Pasture.....	<sup>e</sup> 1,124.0
Reynolds Woods, Coruna, Ind.....	1,000.7		1,147.4
Ellsworth Woods, Ellsworth, Ill.....	994.1		1,059.7
Liebold Woods, Mount Carmel, Ill.....	898.0	Cowpeas.....	1,018.2
Shades, Shades, Ind.....	1,019.1	(f).....	
Real or weighted average.....	<sup>e</sup> 936±3.2		<sup>e</sup> 1,079±2.6
Standard deviation.....	93.62		75.77

<sup>a</sup> 10 determinations unless otherwise noted.

<sup>b</sup> 20 determinations.

<sup>c</sup> 13 determinations.

<sup>d</sup> 12 determinations.

<sup>e</sup> Not included in average, as explained in text.

<sup>f</sup> No comparable field.

<sup>g</sup> Based on all determinations.

When the weights of this constant volume of different soils were tabulated and averaged (Table 1), it was found that the difference between average weights of forest and field soils was 143 g. In other words, the average field soil was 15.3 per cent heavier than the average

forest soil in the upper 9 inches. Since the samples were taken in August and September during an extremely dry period, moisture content was at a minimum in all samples included. Laboratory determinations show slightly higher percentages of moisture in woods than in field soils; therefore, the comparison of woods with field soil is justified on field-weight basis since the difference is slight and would tend to reduce rather than increase any difference between weights.

The Fricke Woods samples, which were not included in the average in Table 1, were the one exception in moisture content. These samples were taken at the end of the summer season after a sudden heavy rain. It is interesting to observe that when the samples were taken on the day following the rain, moisture had penetrated to a depth of 9 inches in the woods, on a 10 per cent slope, whereas in an adjoining field with less slope the penetration of moisture was only 3 inches. The difference was due undoubtedly to superficial run-off in the open. This belief is strengthened by observations of serious erosion damage in the field.

TABLE 2.—*Moisture, specific gravity, and pore space in old-growth forest and field soils*

Soil-sampling site	Capillary water		Hygroscopic water		Apparent specific gravity	
	Forest	Field	Forest	Field	Forest	Field
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		
Myers Woods, Oldenburg, Ind. ....	8.93	3.51	1.030	1.197	1.1801	1.3911
Berkey Woods, Warsaw, Ind. ....	22.25	20.77	1.237	.962	1.2715	1.3857
Rosebrook Woods, Warsaw, Ind. ....	22.25	15.74	1.565	1.172	1.1115	1.2526
Pines Park Woods, Dixon, Ill. ....	17.64	13.12	1.520	1.817	1.1088	1.3252
Bent Tree Woods, Elizabeth, Ill. ....	4.38	5.93	1.537	1.559	1.1061	1.2674
Foley's Woods, Paris, Ill. ....	13.38	7.76	2.372	1.325	1.0175	1.4126
Rhymer Woods, Dongola, Ill. ....	16.27	13.90	1.350	1.115	.9906	1.2782
Turkey Run, Marshall, Ind., No. 1 ..	8.93	5.26	.759	1.262	1.0981	1.3508
Turkey Run, Marshall, Ind., No. 2 ..	7.30	5.26	.840	1.262	1.1142	1.3508
Dillon Woods, Porter, Ind. ....	13.63	13.89	.777	1.094	1.0887	1.1962
Spring Mill Park, Mitchell, Ind. ....	7.99	13.38	1.245	2.825	1.1276	1.2903
Lewis Woods, Williamsburg, Ind. ....	32.63	27.22	2.467	1.660	.8723	1.1424
Brownfield Woods, Urbana, Ill. ....	14.94	8.93	2.290	1.310	1.0698	1.3992
Fricke Woods, Jonesboro, Ill. ....	40.05	13.64	1.380	2.127	.9569	1.3306
Reynolds Woods, Coruna, Ind. ....	13.63	10.86	1.450	1.612	1.1841	1.3925
Ellsworth Woods, Ellsworth, Ill. ....	8.69	12.11	1.872	1.982	1.2298	1.2702
Liebold Woods, Mount Carmel, Ill. ....	13.12	9.09	1.775	.972	1.0672	1.2594
Warren Woods, Three Oaks, Mich. ....	6.16	1.83	.952	.645	1.1102	1.2298

Soil-sampling site	Real specific gravity		Pore space, with percentage excess in forest soil		
	Forest	Field	Forest	Field	Excess
			<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
Myers Woods, Oldenburg, Ind. ....	2.598	2.618	54.57	46.86	16.45
Berkey Woods, Warsaw, Ind. ....	2.585	2.611	50.81	46.93	8.27
Rosebrook Woods, Warsaw, Ind. ....	2.584	2.517	56.98	50.23	13.44
Pines Park Woods, Dixon, Ill. ....	2.607	2.598	57.47	48.99	17.31
Bent Tree Woods, Elizabeth, Ill. ....	2.647	2.614	58.21	51.52	12.99
Foley's Woods, Paris, Ill. ....	2.577	2.566	60.52	44.94	34.67
Rhymer Woods, Dongola, Ill. ....	2.615	2.578	62.12	50.42	23.21
Turkey Run, Marshall, Ind., No. 1 ..	2.620	2.619	58.08	48.42	19.95
Turkey Run, Marshall, Ind., No. 2 ..	2.636	2.619	57.73	48.42	19.23
Dillon Woods, Porter, Ind. ....	2.626	2.609	58.54	54.15	8.11
Spring Mill Park, Mitchell, Ind. ....	2.615	2.703	56.88	52.26	8.84
Lewis Woods, Williamsburg, Ind. ....	2.599	2.626	66.44	56.50	17.59
Brownfield Woods, Urbana, Ill. ....	2.606	2.638	58.95	46.96	25.53
Fricke Woods, Jonesboro, Ill. ....	2.649	2.706	63.87	50.83	25.65
Reynolds Woods, Coruna, Ind. ....	2.608	2.642	54.60	47.29	15.46
Ellsworth Woods, Ellsworth, Ill. ....	2.632	2.642	53.27	51.92	2.60
Liebold Woods, Mount Carmel, Ill. ....	2.626	2.644	59.63	52.37	13.86
Warren Woods, Three Oaks, Mich. ....	2.596	2.671	57.23	53.96	6.06

Table 2 shows the percentage of capillary water, the percentage of hygroscopic water, apparent specific gravity, real specific gravity, and percentage of pore space in forest and field soils, together with the percentage of excess pore space in forest over corresponding field soils.

Dry weights of field soils were calculated by multiplying the average of 10 field weights on each site by the moisture percentage determined on the 500-g sample taken for that site. The 500-g samples were air-dried and the capillary water calculated on the air-dry basis. The air-dry soil was oven-dried and the hygroscopic moisture determined on the oven-dry basis. Real specific gravity was determined by the usual pycnometer method. The volume of the core of soil in each case was 744 cm<sup>3</sup>. Apparent specific gravity was determined by dividing the air-dry soil weight by 744.

Averages of real specific gravities for forest and field soils indicated only very slight differences— $2.613 \pm 0.0032$  (standard deviation, 0.020) for forest soils and  $2.623 \pm 0.007$  (standard deviation, 0.044)

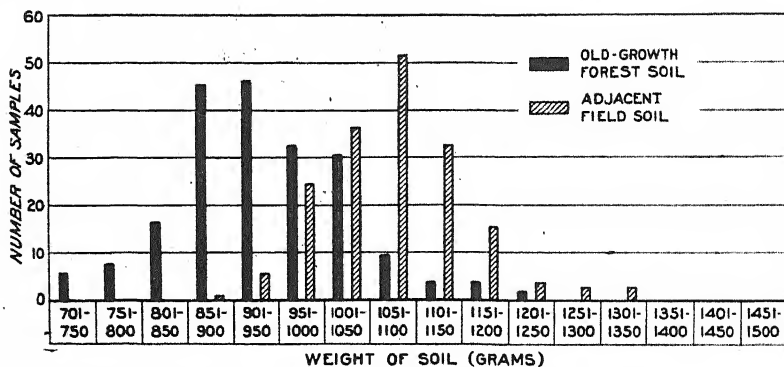


FIGURE 3.—Distribution of 405 samples (0-9.25-inch soil section) of old-growth forest and adjacent field soil

for field soils. The similarity of these figures eliminates serious question as to the possibility of the difference in forest and field soils being caused by difference in the actual weight of the soil material.

When the weights of the constant volume (744 cm<sup>3</sup>) of soil are assembled into groups varying from 701 g to 1,350 g by class intervals of 50, as 701 to 750, 751 to 800, etc., a distinct difference in range of weights is exhibited in forest and field soils. This is shown in Figure 3.

The question naturally arises, to what depth does the increased porosity of forest soils extend? Does the difference occur only at the immediate surface or is it distributed throughout the entire 9-inch profile? This point is significant in the survival of tree seedlings, because if the increased porosity extends to any considerable depth, seedling roots will be able to penetrate much easier and the percentage of survival will be greater. The much mooted question as to difference in water-holding capacities of forested and cultivated areas would be clarified by knowledge of the extent of looseness below the surface.

To settle this question, specific-gravity tests were made the following year in each of three successive 3-inch soil sections in woods and

field on similar old-growth sites.<sup>4</sup> (Table 3.) By driving the 12¼-inch tube into the soil until lengths of 9, 6, and 3 inches remained above the soil surface, columns were obtained of 3.25, 6.25, and 9.25 inches, respectively. The second 3-inch column weight was obtained by subtracting the average weight for the 3.25-inch column in succession from each 6.25-inch column weight. Likewise, the weights of the third 3-inch column were obtained by subtracting the average weight of the 6.25-inch column from the 9.25-inch column weights. By this procedure, the weights obtained were for successive columns of 3.25, 3, and 3 inches.

TABLE 3.—Average weights (grams) of constant soil volumes, on air-dry basis, at three different depths in various old-growth forest areas and in adjacent open fields<sup>a</sup>

Forest soil location	Open areas	0-3.25 inch depth		3.25-6.25 inch depth		6.25-9.25 inch depth	
		Forest	Field	Forest	Field	Forest	Field
Morton Woods, Gallipolis, Ohio.	Pasture-----	251.5	397.1	316.8	316.7	307.3	371.7
Carnahan Woods, Oakwood, Ohio.		191.3	339.1	282.5	364.6	276.3	240.9
Reynolds Woods, Coruna, Ind.		251.3	372.8	251.3	345.5	417.6	337.4
Bent Tree Woods, Elizabeth, Ill.		287.8	364.1	264.6	286.4	298.0	352.1
Sherman Grooms Woods, West Union, Ohio.	Meadow-----	296.6	386.9	309.9	292.9	-----	-----
Beech-maple Woods, Turkey Run, Ind.		241.6	382.4	227.2	373.0	421.1	240.0
Ferris Woods, Fort Ancient, Ohio.	Cornfield-----	309.6	382.1	300.0	272.0	344.3	360.8
Dale Robinson Woods, Bethel, Ohio.		300.0	385.4	335.6	324.9	325.6	331.4
Snyder Woods, Forestville, Ohio.	Wheat field.	241.6	381.1	339.7	357.7	274.0	303.1
Houston Woods, Oxford, Ohio.		340.9	398.5	319.4	318.5	270.0	362.5
Pines Park Woods, Dixon, Ill.		244.4	369.1	273.0	309.0	452.2	416.4
Brownfield Woods, Urbana, Ill.		255.2	400.2	285.0	381.6	394.3	350.4
Rhymer Woods, Dongola, Ill.		226.8	373.2	238.6	308.3	393.1	406.7
Dillon Woods, Porter, Ind.		225.8	347.2	297.9	296.8	404.2	369.6
Myers Woods, Oldenburg, Ind.		268.9	348.9	359.9	392.0	327.2	303.0
Real or weighted average.		202±2.11	375±1.0	293±2.0	329±2.0	350	345
Standard deviation		49.75	29.36	63.59	47.5	-----	-----

<sup>a</sup> Soil volumes were 241.3 cm<sup>3</sup> at middle and lowest depths and 261.4 cm<sup>3</sup> at first horizon. Values are averages of 10 determinations unless otherwise stated.

<sup>b</sup> Average 5 determinations.

<sup>c</sup> Based on all determinations.

The average weights of samples of the surface 3.25 inches were 262 g for forest-soil and 375 g for field-soil samples. This is a difference of 113 g or 43.13 per cent; in other words, the first 3.25 inches of an average field-soil area is on an average, 43.13 per cent heavier than the first 3.25 inches of an adjacent old-growth forest soil. This difference is based on the mineral soil below the litter and duff accumulation. Similarly, the average weight of the 3.25-6.25 inch soil section is 12.29 per cent greater for the field than for the woods soil. Figures 4 and 5 show the range and distribution of weights of the two lower soil sections. In the lowest section the difference is negligible, and

<sup>4</sup> The original soil weights of the first year's work are given on a field-weight basis, whereas the weights of the second year's work were on an air-dry basis. This will not alter the comparative relationship between forest and field soils, but should be taken into consideration if the actual weights of the first year's work are compared with those obtained the second year.

therefore no attempt was made to show graphically the distribution of weight classes.

From the foregoing comparisons it may be seen that the greatest difference in porosity between old-growth woods soil and cultivated soil is in the first 6-inches. But although the lower section shows no difference by apparent specific gravity measurements, water-absorp-

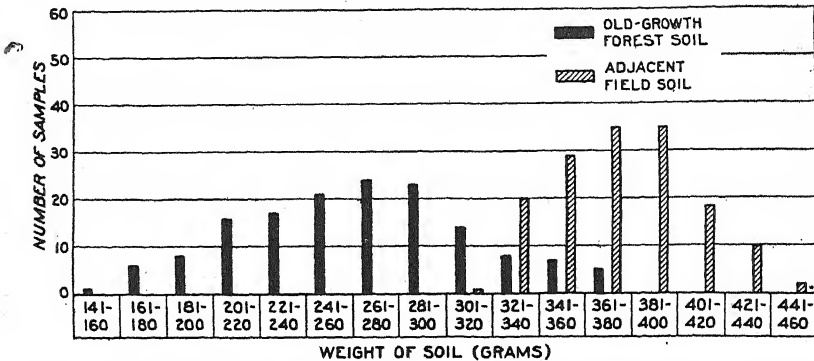


FIGURE 4.—Distribution of 300 samples (0-3.25 inch soil section) of old-growth forest and adjacent field soils, by weight classes

tion experiments show that the lower section at a depth of 8 inches absorbs water much more rapidly in woods than in field soil.

#### WATER ABSORPTION IN OLD-GROWTH FOREST SOILS

As a corollary to the measurements of apparent specific gravity, a series of absorption tests were made on virgin forest-soil areas and adjacent fields. Five brass tubes 2 by 12 inches were used. The tubes were sharpened on one end and forced into the soil to a con-

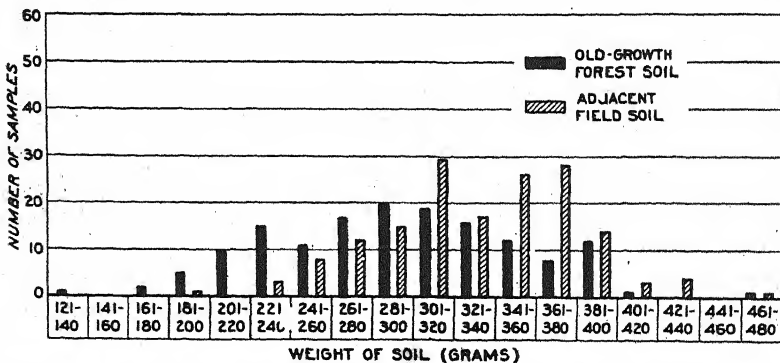


FIGURE 5.—Distribution of 300 samples (3.25-6.25 inch soil section) of old-growth forest and adjacent field soils, by weight classes

stant depth of 3 cm. This depth proved sufficient to insure that the water would not flow up around the end.

Absorption was measured at three different depths; 1, 3, and 8 inches. In the first depth, the litter or duff layer was removed. In the second or 3-inch depth, a trench 2 inches deep was dug before the tubes were driven in. In the lower depth, a trench 7 inches deep was



dug. This procedure placed the bottoms of the brass tubes 1, 3, and 8 inches below the soil surface.

The three tubes were kept filled to the top by means of five graduated cylinders each containing 800 cm<sup>3</sup>. At the end of 10 minutes the water remaining in each graduate was measured. This volume in each case added to the known constant volume of the corresponding brass tube, less the volume of the small column of soil at the bottom of the brass tube, and subtracted from 800, gave the amount absorbed. In the case of very rapid absorption, a smaller unit of time was used, and in the case of very slow absorption, the small amount of water was determined by measuring the level to which the water sank during the time interval, and by calculating its volume from the known diameter of the brass tube.

TABLE 4.—*Rates of water absorption in old-growth and open-field soils, in cubic centimeters per minute, at 1-inch, 3-inch, and 8-inch depths*<sup>a</sup>

Forest soil location	Open area	1-inch depth		3-inch depth		8-inch depth	
		Forest	Field	Forest	Field	Forest	Field
Morton Woods, Gallipolis, Ohio.....	Pasture.....	Cc 165.8	Cc 0.8	Cc 6.0	Cc 1.2	Cc 1	Cc 1
Houston Woods, Oxford, Ohio.....		11.0	1.2	1.2	1.4	2.4	5.2
Reynolds Woods, Coruna, Ind.....		89.6	5.0	18.4	12.6	8.6	5.8
Bent Tree Woods, Elizabeth, Ill.....		370.6	3.4	10.6	3.0	1.4	5.8
Sherman Grooms Woods, West Union, Ohio.....		187.8	2.0	11.0	9.0	2.0	1.2
Ferris Woods, Fort Ancient, Ohio.....	Cornfield.....	12.0	1.2	4.0	1.4	.8	2.2
State Sanitarium Woods, Mount Vernon, Ohio.....		60.2	5.2	21.8	2.4	12.2	3.2
Poplar Cove Woods, Haverhill, Ohio.....	Wheat stubble.....	144.0	3.6	10.8	4.6	1.2	2.0
Dale Robinson Woods, Bethel, Ohio.....		105.6	.6	8.0	5.6	1.4	.4
Snyder Woods, Forestville, Ohio.....		425.8	4.0	6.0	3.4	3.8	7.4
Pines Park, Dixon, Ill.....		822.4	4.6	24.2	2.4	5.6	3.0
Brownfield Woods, Urbana, Ill.....		175.4	4.6	139.8	3.0	4.8	2.4
Rhymer Woods, Dongola, Ill.....		282.8	1.8	446.2	5.8	15.0	2.4
Dillon Woods, Porter, Ind.....		423.8	5.8	236.0	4.8	5.6	4.4
Myers Woods, Oldenburg, Ind.....		175.6	2.6	92.8	7.6	2.2	.6
Daniel Mosier Woods, Spellacy, Ohio.....		127.6	11.0	-----	-----	27.6	7.8
Beech-maple Woods, Turkey Run, Marshall, Ind.....		194.8	9.8	113.8	9.2	14.4	9.4
Keffer Woods, Mifflin, Ohio.....	Meadow.....	84.6	4.6	-----	-----	50.2	3.4
Chas. Armstrong Woods, Loudonville, Ohio.....		32.6	3.8	-----	-----	15.0	5.4
Average (rounded).....	-----	205	4	72	5	9	4

<sup>a</sup> Each figure represents the average of 5 determinations.

Table 4 shows the average absorption per minute at the three depths in woods and field. The ratios of water absorption per minute at the 1, 3, and 8 inch depths in forest and field were 205 to 4, 72 to 5, and 9 to 4, respectively.

This method of measuring absorption rates must be considered only relative. Rainfall water penetrates only vertically downward, the water of adjacent areas prevents the horizontal movement possible from the tubes. Horizontal absorption is, however, roughly proportional to porosity; consequently, on a comparative basis, the procedure is justifiable. The relative rates of absorption in forest and field strengthen the conclusion drawn from the apparent specific gravity measurements, namely, that forest soils are more porous than field soils. (Fig. 4.)

On 36 second-growth areas in Ohio, representing nearly all major soil areas of the State, 718 specific-gravity tests were made in the

0-9.25 inch horizon. (Table 5.) The average weight of 744 cm<sup>3</sup> of dry soil was, for the second-growth woods, 873 g and for the adjacent field soil 1,023 g. Figure 6 shows the distribution of weight classes as between the forest and open soils. The significant fact about this comparison is that the second-growth woods soil is much more porous than the corresponding field soil. Apparently any deleterious effect of the cutting on soil tilth has been remedied by the closing in of the crowns and the prompt reestablishment of a litter cover.

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TABLE 5.—Average weights of constant soil volumes (744 cm<sup>3</sup>) to a depth of 9.25 inches of ungrazed second-growth forest areas and adjacent open fields or heavily grazed woods<sup>a</sup> in Ohio

Forest-soil location	Ungrazed forest soil	Open or grazed area	
		Soil sample	Description
	<i>Grams</i>	<i>Grams</i>	
Richmondale.....	935.1	1,062.2	Abandoned field.
Athens.....	<sup>b</sup> 947.1	1,002.2	
Idaho.....	880.7	1,016.6	
Indian Springs.....	850.5	1,015.3	
Bentonville.....	881.6	1,033.2	Meadow.
Russellville.....	945.4	1,050.5	
Meeker.....	<sup>b</sup> 817.2	1,044.7	
Brighton.....	1,008.4	1,128.2	
Mutual.....	1,000.4	1,159.5	
Columbus.....	971.5	1,032.3	
Johnsville.....	872.9	1,039.3	
Vermilion.....	739.8	936.4	
West Union.....	944.5	947.0	
Bethel.....	898.1	1,062.2	Pasture.
Batavia.....	928.4	1,008.5	
Montgomery.....	836.5	1,045.7	
Circleville.....	833.4	1,067.4	
Do.....	935.2	1,108.2	
Marion.....	918.7	1,164.2	
Mansfield.....	769.7	1,043.1	
Mount Vernon.....	824.3	999.1	
Sunbury.....	895.1	975.2	
Marysville.....	910.8	998.5	Heavily grazed woods.
Alta.....	766.2	1,024.9	
Clyde.....	885.0	1,134.7	
Bucyrus.....	803.0	993.6	
Marengo.....	853.9	996.8	
Clarksville.....	931.3	950.6	
Marion.....	832.2	946.5	
Upper Sandusky.....	837.9	969.1	
Marion.....	906.6	993.9	
Galion.....	679.7	837.8	Typical examples of grazed and ungrazed woods. <sup>c</sup>
Mansfield.....	827.1	1,038.7	
Lexington.....	874.6	915.1	
Mount Gilead.....	882.7	1,043.1	
Mount Vernon.....	810.8	1,030.5	
Real or weighted average <sup>e</sup> .....	873±2.5	1,023±2.4	
Standard deviation <sup>d</sup> .....	97.9	81.1	

<sup>a</sup> Averages of 10 determinations unless otherwise noted.

<sup>b</sup> Average of 9 determinations.

<sup>c</sup> Average of 9 typical examples, ungrazed woods, 843±4.9 g (S. D. 68.7), grazed woods, 969±4.5 (S. D. 63.0).

<sup>d</sup> Based on all determinations.

Where grazing has been practiced, soil porosity has been much reduced. Apparently conservative cutting in this region preserves soil porosity. Even where cutting has been severe, the reestablishing of a litter cover has prevented excessive soil deterioration. This fact is at once apparent when a comparison is made between the differences in weights of old-growth forest soil and adjacent field soils, and the differences between second-growth woods soil and adjacent field soil. They are 144 and 150 g, respectively, for the 9.25-inch column 2½ inches in diameter. This demonstrates that soil porosity is main-

tained in second-growth woods soil. This general conclusion is strengthened when it is appreciated that the sites were selected at random and represented oak-hickory, beech-maple, pure beech, pure oak, mixed mesophytic, and elm-ash-maple forest types. The soils of the various sites represented glaciated and unglaciated areas, limestone and sandstone understrata, Wisconsin and Illinoian glaciation, flat and rolling topography, well drained and poorly drained sites. The second-growth woods in every case showed a lighter soil than did the field areas adjacent. The second-growth woods were of all ages from 10 to 70 years. Cutting had been severe in many instances, but in practically all cases the crown cover had filled in the vacant spaces.

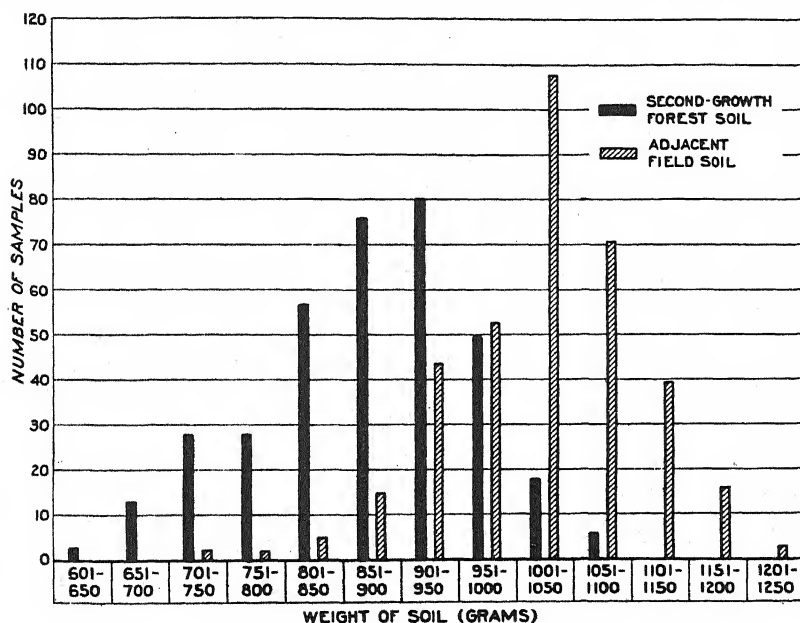


FIGURE 6.—Distribution of 718 samples (0-9.25 inch soil section) of second-growth forest and adjacent field soils, by weight classes

#### SOIL POROSITY OF GRAZED AND UNGRAZED WOODS

From the list of second-growth sites, nine were selected as typical of grazed and ungrazed woods. The grazed woods were in the last stages of forest degradation—that is, there were only a few old trees, no reproduction, and a ground cover of weeds, grass, and sod. Table 5 gives the weights of the 0-9.25 inch soil section. Figure 7 presents a comparison of grazed and ungrazed areas, as shown in the distribution of weight classes. The average weight of the 744 cm<sup>3</sup> core of soil on the basis of 180 samples of air-dry soil was 843 g for the ungrazed, and 969 g for the grazed areas, a difference of 126 g. This, interpreted in percentages, means that a grazed woods soil in the 0-9.25 inch section is 15 per cent heavier than the corresponding soil section in an adjacent ungrazed area. Excessive grazing not only prevents tree reproduction but also makes forest exhaustion inevitable through compaction of the soil. The grazed soil is harder, holds less moisture, has poorer aeration, and its general tilth is much deteriorated.

# SOIL POROSITY IN FOREST PLANTATIONS AND OPEN FIELDS

The final angle of the soil porosity question deals with forest plantation sites. Does soil porosity return to old fields after planting? Thirteen plantations were measured for soil porosity and compared to

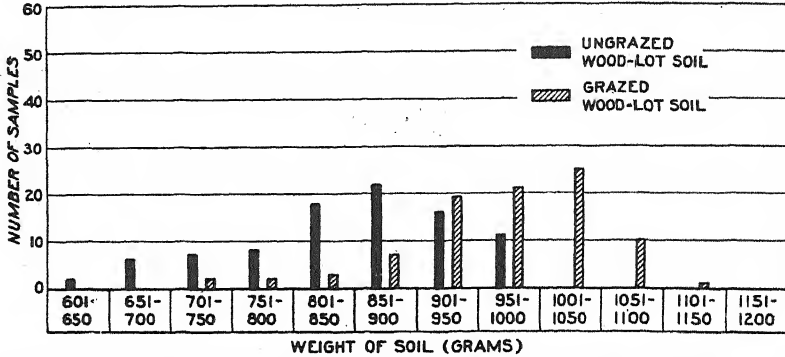


FIGURE 7.—Distribution of 180 samples (0-9.25 inch soil section) of grazed and ungrazed wood-lot soils, by weight classes

adjacent field sites. (Table 6.) The difference in average weight of 744 cm<sup>3</sup> cores of forest plantation soil and field soil was 128 g, or a 13.6 per cent greater weight for field soil than for adjacent plantation soil. Figure 8 shows the distribution of weight classes. The lighter

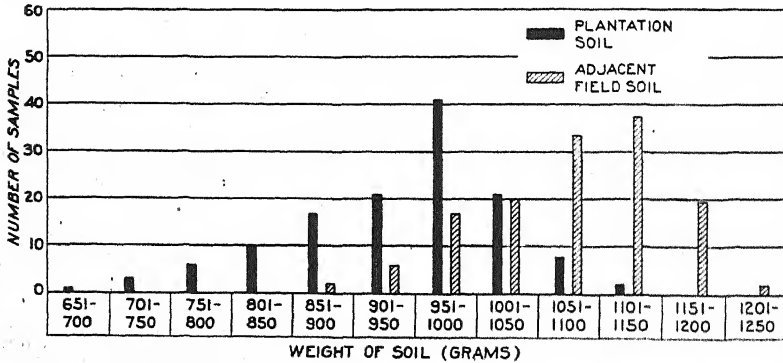


FIGURE 8.—Distribution of 259 samples of plantation and adjacent field soils, by weight classes

average for plantation soil represents a gain in porosity since planting. Field observations show that as soon as a litter is established under a stand the soil becomes more porous. The observation was made that the soil of conifer plantations gained more in porosity in a given time than did that of the hardwoods.

TABLE 6.—Average weights <sup>a</sup> of constant volumes of soil (744 cm<sup>3</sup>) to a depth of 9.25 inches in forest plantations and adjacent open fields

Location	Plantation			Open area	
	Species <sup>b</sup>	Age	Soil sample	Description	Soil sample
		Years	Grams		Grams
Fremont, Ohio.....	Hardy catalpa.....	29	1,006.0	Pasture.....	1,084.5
	Scotch pine and Norway spruce.....	19	927.4	do.....	1,081.4
Wooster, Ohio.....	Northern white pine.....	20	950.8	do.....	1,081.4
	Norway spruce.....	14	937.7	do.....	1,081.4
	Beech.....	15	993.1	do.....	1,081.4
	Norway pine and Norway spruce.....	18	926.7	do.....	1,081.4
Colebrook, Ohio.....	do.....	15	792.2	Abandoned field.....	929.4
Athens, Ohio.....	Norway pine.....	12	980.3	Cornfield.....	1,033.9
	Corsican pine.....	12	1,065.5	do.....	1,083.5
Urbana, Ill.....	Bur oak.....	42	834.3	do.....	1,134.4
	European larch.....	59	982.3	do.....	1,134.4
Athens, Ohio.....	Northern white pine.....	23	972.7	Sod.....	1,088.4
Marietta, Ohio.....	Norway pine.....	17	886.3	do.....	1,021.4
Real or weighted average.....			943±5.1		1,071±4.2
Standard deviation.....			85.64		70.97

<sup>a</sup> Averages of 10 determinations unless otherwise noted.<sup>b</sup> Botanical names of species are as follows: Hardy catalpa (*Catalpa speciosa* Ward); Scotch pine (*Pinus sylvestris* L.); Norway pine (*P. resinosa* Sol.); northern white pine (*P. strobus* L.); Corsican pine (*P. nigra* var. *poiretiana* Asch. and Graebn.); Norway spruce (*Picea abies* Karst.); European larch (*Larix decidua* Mill.); beech (*Fagus grandifolia* Ehrh.); bur oak (*Quercus macrocarpa* Mich.).<sup>c</sup> Average of 9 determinations.

## WATER ABSORPTION IN FOREST-PLANTATION SOIL

Water absorption tests in plantation and adjacent field soils demonstrated that plantation soils have a much greater absorption capacity. (Table 7.) The average for the plantation sites at the 1-inch depth was 107 cc per minute and for the field site at 1-inch depth, 8 cc. At the 8-inch depth, the averages were 13 and 3 cc per minute for the plantation and field sites respectively. These differences in absorption capacity justify the weight differences recorded and show a gain in soil porosity for the plantation since planting.

TABLE 7.—Average rate of water absorption in cubic centimeters per minute in forest plantation and adjacent field soil<sup>a</sup>

Location	Plantation				Open area		
	Species <sup>b</sup>	Age	Soil sample		Description	Soil sample	
			1-inch depth	8-inch depth		1-inch depth	8-inch depth
		Years	Cc	Cc		Cc	Cc
	Scotch pine and Norway spruce.....	19	32.6	4.6	Pasture.....	5.2	4.2
Wooster, Ohio.....	Northern white pine.....	20	21.0	14.6	do.....	5.2	4.2
	Beech.....	15	81.4	3.2	do.....	5.2	4.2
	Norway pine and Norway spruce.....	18	69.8	3.4	do.....	5.2	4.2
Colebrook, Ohio.....	Norway spruce.....	15	367.4	1.0	Abandoned field.....	44.8	5.0
Athens, Ohio.....	Norway pine.....	12	8.6	2.4	Cornfield.....	4.4	1.2
	Corsican pine.....	12	5.6	6.2	do.....	4.4	2.8
Urbana, Ill.....	European larch.....	59	521.8	69.8	do.....	4.6	2.4
	Bur oak.....	42	42.4	30.4	do.....	4.6	2.4
Athens, Ohio.....	Northern white pine.....	23	13.8	2.6	Sod.....	3.6	2.8
Marietta, Ohio.....	Norway pine.....	17	7.8	1.2	do.....	2.0	1.6
Average (rounded).....			107	13		8	3

<sup>a</sup> Each value is the average of 5 determinations.<sup>b</sup> For botanical names, see Table 6, footnote b.

## FUNCTIONS OF FOREST LITTER

The problem of forest soils is inseparably connected with forest litter, and the proper production and retention of adequate quantities and kinds of forest litter is inseparably connected with good silvicultural practice. This relationship of litter to good forestry methods may be appreciated better when the many functions of litter are considered. The accumulation of litter depends upon three major factors: Moisture, temperature, and ease of decomposition. Given a high average temperature and a sufficient supply of moisture, leaf litter decomposes very rapidly. In the Tropics, for instance, leaf litter does not accumulate to any great extent because of the high average temperature and high humidity. Indeed, the oxidation is so rapid that not only does the leaf litter disappear, but the soil itself is oxidized to a considerable depth, a condition which gives rise to the so-called laterite or red soil. In the Temperate Zone the accumulation of leaf litter is more rapid and in the sub-Arctic Zone the accumulation of litter becomes so great that it may actually be a deterrent to tree growth. It locks up the plant food in the rather tight layer of raw vegetative material, the rate of decay of which is not rapid enough to return plant food to the soil.

In the Central States region the accumulation of litter is small compared with that in the northern spruce belt. In fact, it is seldom more than 1 inch deep. The leaf fall decomposes rapidly and adds its fertility to the soil. The forest litter is, however, of great importance in this region even though it does not accumulate to a great depth. It serves as an absorbent, acts as a mulch or insulation against rapid evaporation, prevents compaction of surface soil by impact of rain, decomposes and furnishes plant food, and serves as a medium for microbiological activities.

Structure of forest soil has been developed through long periods of time. Root penetration, expansion, and contraction, with changes in temperature and moisture content, and activities of worms, insects, and animals, make soil porous. The forest with its protective soil cover of leaf litter and its deep root system is a builder and preserver of soil porosity. The penetration of roots and their eventual decay leave the soil interpenetrated with tubelike cavities. (Fig. 9.)

The process of cropping, on the other hand, ultimately decreases soil porosity and favors erosion. For example, the increased porosity brought about by freezing and thawing is preserved in forest soils by the covering of litter, but in field soils it is largely lost in summer by impact of rain and silting and alluviating action of percolating waters. When the Central States region was settled originally, both good and poor lands were cleared and cultivated. The accumulation of organic matter in the form of incorporated and unincorporated forest litter afforded a temporary supply of plant food, and even the poorer, shallow soils produced well for a generation. Then this supply became exhausted and the land ceased to pay a reasonable return for its cultivation. As the organic matter oxidized and plant food leached away, the soil became increasingly unproductive. The impoverishment under agricultural use of originally fertile forest soil became a major factor in the growth of land abandonment.

The following is quoted (6, p. 4) from a report of a recent economic survey of Vinton County, Ohio:

There are 263,680 acres of land in the county. In 1880 the area of land in farms was 230,000 acres; by 1910, this had declined to 216,000 acres, and by 1930, to 152,000 acres, or 58.8 per cent of the total land area. In 1930 the area of improved land in the county was less than one-half that in 1900, and the number of farms in the county has decreased from 2,089 in 1900 to 1,823 in 1910 and to 1,075 in 1930.

Such a record needs no interpretation; it speaks deforestation, abandonment, and erosion.

#### DISCUSSION AND CONCLUSIONS

Under virgin conditions an equilibrium has been reached—the kinds and quantities of litter added to the soil do not vary much from season to season and the rate of decomposition has reached a norm.

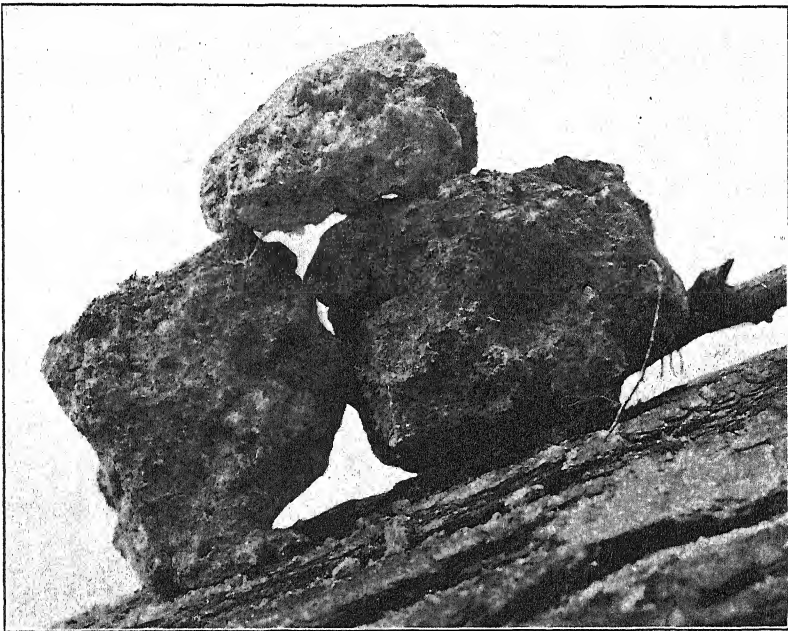


FIGURE 9.—Old-growth forest soil, showing spongelike appearance due to penetration and decay of roots

The tree species best fitted to produce on the site have become established; theoretically, optimum conditions exist for that site. When cutting is done, certain species are usually selected, and the litter equilibrium is thereby disturbed. More sunlight is admitted, the ground cover changes; but after a period of years the crown cover closes in and another equilibrium is established.

The porosity of old-growth forest soil in the upper 9 inches is from 10 to 20 per cent greater than that of adjacent cultivated soil. The difference may be as much as 30 per cent in certain areas. The difference is greatest in the first 3 inches, decreases in the second, and becomes small in the third 3 inches. The method used in this investigation had a rather great limit of error, and although the average weights of forest and field soil were found to be about the same in the

6.25- to 9.25-inch horizon, observation confirmed by absorption tests show that there is a difference in porosity even at a 9-inch depth. For example, forest soils at a depth of 8 inches absorbed water more than twice as fast as did field soils at the same depth.

Not only are old-growth forest soils porous, but many of those examined in this study were also found to be platy in structure. (Fig. 10.) A platy soil has greater water-holding capacity than one

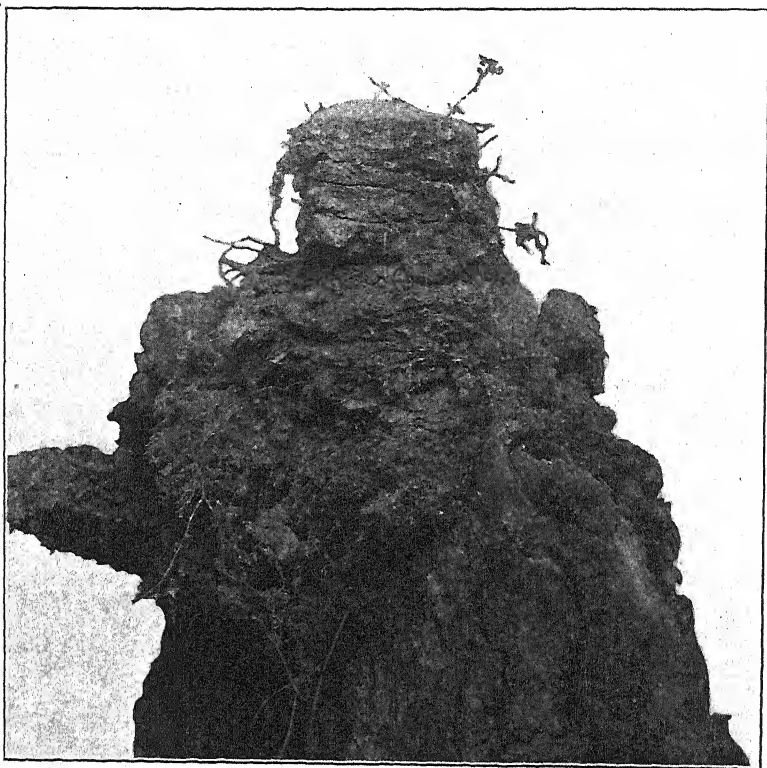


FIGURE 10.—A platy forest soil

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with no structure or with vertical cleavage. Water flows out into the horizontal cleavage planes and is thereby held in the soil in greater quantity than it would be if it were free to flow downward through cracks.

The effects of greater soil porosity in the forested areas are immediately apparent. When one considers that absorption from tubes is over 50 times as fast in old-growth forest soil at 1-inch depth as in cultivated fields at the same depth, the relative amounts of run-off in the respective sites may be visualized. While it is true that there was horizontal absorption in the case of the tubes, it must also be observed that even with horizontal absorption the amount of water taken up in field soil was extremely low. In many cases only a few cubic centimeters were absorbed, whereas in the woods the water ran into the soil with great rapidity. From the standpoint of applying the data to actual rainfall, it is at once clear that there would usually be consid-



erable run-off from even a  $\frac{1}{2}$ -inch precipitation in the field areas, whereas a forest soil would have no difficulty in absorbing such a rain. Even a sudden rain of an inch or more, which is unusual in this region, would ordinarily be absorbed in a dry forest soil.

Virgin forests, except as a valuable means of interpreting optimum growth conditions, are of no concern in the Central States region. The virgin woods are gone; only second-growth and abandoned fields remain. One point is clearly established in respect to second-growth woods and that is the retention of soil porosity. Careful cutting and consequent opening up of the stand does not destroy soil porosity, provided the forest cover is quickly reestablished. A comparison of the weights of soil columns from second-growth woods and fields with those from old-growth forests and fields proves this point. Where the litter has not been destroyed by fire or grazing, the second-growth woods soil retains its virgin porosity to a surprising degree. The crown cover, of course, closes in after cutting and the litter produced reaches an equilibrium again.

The retention of soil porosity in second-growth woods emphasizes the prime importance of fire protection. Given proper protection and good silvicultural management in the way of selective cutting, practically all of the second-growth areas will retain their productive capacity.

What has been said regarding fire protection applies with equal force to overgrazing. Grazing is very injurious to soil structure. Compaction accompanies grazing and compaction means smaller water-holding capacity, poorer aeration, and general decrease in tilth and productivity. Trees become stag-headed where grazing is continued, litter largely disappears, sod forms, and the woods grow thin and finally disappear. Moderate cutting alone does not destroy soil porosity. The study of second-growth areas establishes the fact that soil porosity of old-growth areas is largely retained in second-growth woods provided grazing has not been practiced.

A problem of great concern in this Central States region is that of abandoned land and soil erosion. Six and a half million acres of land are considered idle. Most of this area was once wooded; now its fertility is largely exhausted by cultivation and erosion. The rule in the past has been to plant on such lands the species which formerly grew there naturally or to trust to luck that the area would become reseeded, without realizing that the cleared and abandoned field soil might not be capable, in its condition of deterioration, of favoring the reestablishment of the original tree growth. Some difficulty has been experienced in getting hardwood plantations established on such lands; conifer plantations, especially of pine, are as a rule more successful. Hardwoods, with the exception of black locust, are not so able to survive extremes of temperature and moisture deficiency, such as often occur in worn-out soil.

Conifers offer a means of bridging over the unfavorable period between abandonment and reforestation. On some lands planting is necessary to develop a cover. Before initiating a planting program, however, it is well to observe the condition of the soil. If the soil lacks structure, bakes badly, and is subject to severe drying in summer, hardwoods will probably fail. Many denuded areas will reseed eventually and regenerate naturally where there are seed trees. Such abandoned areas gradually seed in and establish a stand; then,

as a litter cover develops, the soil improves and severe extremes of temperature and moisture are obviated, paving the way for hardwoods. Such a transition is, however, often very slow.

In brief, from the results obtained in the studies here detailed, the following conclusions may be drawn:

Forest soil is much more porous than field soil.

Forest soil porosity is greatest in the upper 6 inches.

Porosity differences in forest soil and field soil are caused more by soil structure than by organic matter content, as shown by real specific gravity measurements.

Forest soil absorbs water much more rapidly than does field soil.

Water absorption is most rapid in forest soil at 1-inch depth and gradually decreases downward.

Ability of bare soil to absorb water is less at the surface than at 3-inch depth. A crust forms on the surface from impact of rain.

Where the forest cover is adequately maintained, second-growth forest soil does not lose its porosity unless grazing has been practiced to excess or the litter has been destroyed by fire.

Forest soil shows great loss of porosity when overgrazed.

Plantations on cultivated fields regain a large degree of soil porosity in 20 to 25 years as evidenced by soil weight and ability to absorb water.

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# INHERITANCE OF FUSARIUM RESISTANCE IN BRUSSELS SPROUTS AND KOHLRABI<sup>1</sup>

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## INTRODUCTION

The inheritance of resistance in both wild and cultivated cabbage (*Brassica oleracea* L. and *B. oleracea capitata* L.) to the yellows disease caused by *Fusarium conglutinans* Woll. was found by Walker<sup>2</sup> to be based upon a single dominant Mendelian factor. Moreover, it has been pointed out by Walker and Wellman<sup>3</sup> that the subspecies of *B. oleracea* other than cabbage are also susceptible to the yellows organism and that considerable differences in percentages of susceptible individuals are found among the commercial varieties of any one of the subspecies. The present paper is the report of a study of the inheritance of resistance to yellows in two of the subspecies of *B. oleracea*, namely, Brussels sprouts (*B. oleracea gemmifera* DC.) and kohlrabi (*B. oleracea caulorapa* DC.).

## METHODS

The methods employed in this study were similar to those reported in the studies on cabbage.<sup>4</sup> Initial selections of resistant material were made from commercial varieties of Brussels sprouts and kohlrabi which had been grown on soil heavily infested with yellows. The selected material was brought to flower in the greenhouse, where controlled pollination was practiced.

Field trials were conducted in Kenosha County, Wis., on soil which was known to be thoroughly infested with the yellows organism. Seed was planted in seed beds and the resulting seedlings were transplanted to the trial plot. As symptoms of the disease appeared, with the advent of favorable soil temperatures, the diseased plants were permanently marked by means of bamboo stakes. Greenhouse trials of many of the plant progenies were conducted under controlled conditions of soil temperature in soil into which the yellows organism had been previously introduced. In the results as presented no distinction has been made in regard to the severity of the infection. Plants classified as diseased include those that showed any degree of yellows infection, ranging from symptoms so slight as to permit eventual recovery to those so severe as to cause the death of the plant. The delayed attack of the organism or its mild pathogenicity in some plants as contrasted with very severe forms of the disease in other

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<sup>2</sup> WALKER, J. C. INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE. Jour. Agr. Research 40: 721-745, illus. 1930.

<sup>3</sup> ——— and WELLMAN, F. L. A SURVEY OF THE RESISTANCE OF SUBSPECIES OF BRASSICA OLERACEA TO YELLOWS (FUSARIUM CONGLUTINANS). Jour. Agr. Research 37: 233-241, illus. 1928.

<sup>4</sup> WALKER, J. C. Op. cit. (See footnote 2.)

plants, can not be fully explained. As stated elsewhere,<sup>5</sup> these differences may depend on factors as yet unrecognized.

### EXPERIMENTAL RESULTS

Commercial lots of Long Island Improved Brussels sprouts and White Vienna kohlrabi were grown on the yellows plot for three successive years. The results given in Table 1 show that the percentage of resistant plants was much higher in each of these varieties than in a commercial susceptible cabbage variety grown for comparison. Selections of Brussels sprouts and kohlrabi were made from plants that remained free from the yellows disease throughout the season of 1928. After the customary period in storage the plants were brought to the greenhouse and came to flower during the following winter. The data on inheritance of *Fusarium* resistance in each of the two subspecies will be considered separately.

TABLE 1.—Occurrence of yellows in field trials of commercial varieties of Brussels sprouts, kohlrabi, and cabbage

Common name and variety	Year	Plants grown on yellows plot			
		Total	Healthy	Diseased	
		Number	Number	Number	Per cent
Brussels sprouts, Long Island Improved.....	1928	76	67	9	12
	1929	40	31	9	23
	1930	104	80	24	23
Total.....		220	178	42	19
Kohlrabi, White Vienna.....	1928	75	46	29	39
	1929	50	40	10	20
	1930	84	71	13	15
Total.....		209	157	52	25
Cabbage, Danish Ballhead.....	1928	549	10	539	98
	1929	1,095	62	1,033	94
	1930	1,277	52	1,225	96
Total.....		2,921	124	2,797	96

### INHERITANCE OF RESISTANCE IN BRUSSELS SPROUTS

Of the Brussels sprouts plants selected in the field, three (BS-3, BS-4, BS-6) were brought to flower in the greenhouse, and progenies from self-pollination were tested in the field and in the greenhouse in 1929. The results show (Table 2) that plant BS-3 was homozygous for resistance, whereas the progenies from BS-4 and BS-6 segregated into resistant and susceptible classes in a ratio approximating 3 resistant to 1 susceptible. These data indicate that resistance was inherited in a manner similar to that noted for cabbage.<sup>5</sup>

<sup>5</sup> WALKER, J. C. Op. cit. (See footnote 2.)

TABLE 2.—Occurrence of yellows in progenies from self-pollination of three resistant Brussels sprouts plants selected from a commercial strain of Long Island Improved variety, 1929

Genotype class	Progeny No.	Tested in—	Number of plants		Deviation	Probable error	Dev. P. E.
			Healthy	Diseased			
<i>RR</i> .....	BS-3s.	{Field.....	21	0	0	-----	-----
		{Greenhouse.....	33	0	0	-----	-----
	Total.....		54	0	0	-----	-----
<i>Rr</i> .....	BS-4s.	{Field.....	12	5	.75	±1.20	0.62
		{Greenhouse.....	13	3	1.00	±1.17	.85
	BS-6s.	{Field.....	9	3	0	±1.01	0
		{Greenhouse.....	13	14	7.25	±1.52	4.77
	Total.....		47	25	7.00	±2.48	2.82
	Calculated (3:1).....		54	18			

Plants BS-3 and BS-4 were also crossed with a plant (C-131) from a line of Copenhagen Market cabbage which had previously been found to be homozygous for susceptibility. As a test of susceptibility, seed from self-pollination of plant C-131 was planted in the yellows plot. Of the 20 plants tested, all succumbed to yellows. The results of trials in 1929 with crosses between Brussels sprouts and cabbage are given in Table 3. That BS-3 was a homozygous resistant plant was confirmed by the fact that the hybrid progeny was completely resistant. On the other hand, the hybrid progeny from the supposedly heterozygous plant (BS-4) segregated in a ratio approximating 1 resistant to 1 susceptible.

TABLE 3.—Occurrence of yellows in hybrid progenies from crosses between a homozygous susceptible cabbage plant (C-131) and two resistant Brussels sprouts plants, one (BS-3) homozygous for resistance, the other (BS-4) heterozygous for resistance, 1929

Cross	Tested in—	Number of plants		Deviation	Probable error	Dev. P. E.
		Healthy	Diseased			
BS-3 × C-131.....	{Field.....	21	0	0	-----	-----
		25	0	0	-----	-----
	Total.....	46	0	0	-----	-----
BS-4 × C-131.....	{Field.....	14	15	.50	±1.82	0.27
		9	21	6.00	±1.85	3.24
	Total.....	23	36	6.50	±2.59	2.51
	Calculated (1:1).....	29.5	29.5			

From the field trials of the  $F_1$  hybrids in 1929, material was saved from BS-3 × C-131 ( $RR \times rr$ ) and brought into the greenhouse. These plants, all of which were expected to be heterozygous ( $Rr$ ) for resistance, were given numbers (BC-2, BC-3, etc.). Self-pollination was practiced on these plants, as well as emasculation and back crossing with plants from a homozygous susceptible cabbage line of Smith Pride variety (SP-5 and SP-9). Table 4 shows the reaction to the yellows organism, in the field and in the greenhouse, of the  $F_2$  progenies resulting from self-pollination of the  $F_1$  hybrid plants. In the field

trials segregation was very close to the expected ratio of 3 resistant to 1 susceptible. In the greenhouse trials the number in the susceptible class was appreciably below that expected, probably because of the failure of the soil temperature to maintain a level sufficiently high for disease expression, as discussed in a paper by Walker and Smith.<sup>6</sup>

TABLE 4.—Occurrence of yellows in  $F_2$  progenies from a cross between a homozygous resistant Brussels sprouts plant (BS-3) and a homozygous susceptible cabbage plant (C-131)

Progeny No.	Tested in—	Number of plants		Deviation	Probable error	Dev. P. E.
		Healthy	Diseased			
BC-2s	Field	59	14	4.25	±2.50	1.70
	do. "	30	10	0	±1.85	0
	Greenhouse	176	48	8.00	±4.37	1.83
BC-4s	Field	24	11	2.25	±1.73	1.30
	Greenhouse	31	8	1.75	±1.82	.96
BC-5s	Field	26	9	.25	±1.73	.14
	Greenhouse	93	16	11.25	±3.05	3.69
BC-6s	Field	9	2	.75	±.97	.77
	Greenhouse	27	8	.75	±1.73	.43
BC-8s	Field	126	34	6.00	±3.69	1.63
	Greenhouse	14	6	1.00	±1.31	.76
BC-9s	Field	24	8	0	±1.65	0
	Greenhouse	12	4	0	±1.17	0
BC-10s	Field	5	3	1.00		
BC-11s	Field					
Total in field		218	71	1.25	±4.97	.25
Total in greenhouse		438	110	27.00	±6.84	3.95
Grand total		656	181	28.25	±8.45	3.34
Calculated (3:1)		628	209			

\* Test made in 1931; other tests were made in 1930.

The progenies from back crosses between the  $F_1$  hybrid plants and homozygous susceptible cabbage were also tested in the field and in the greenhouse. The field tests include trials made in 1930 and in 1931. As indicated in Table 5, the number of diseased plants in the field trials was considerably smaller than the number expected. This discrepancy may be explained, partly at least, by the fact that the seed bed in 1930 was inadvertently made on soil in which the yellows organism was present. Consequently, part of the susceptible class was eliminated in the seed bed and fewer individuals of that class were transplanted and considered in the field trial. In view of this possible explanation for the unusually small number of individuals in the susceptible class, certain progenies (BC-3 × SP-5, BC-4 × SP-5, and BC-11 × SP-9) were tested in the field the following year. The segregation in the second field trial of these progenies was in general closer to the expected 1 to 1 ratio. In 1930 the three progenies showed 96 healthy and 46 diseased plants, whereas in 1931 the same progenies showed 147 healthy and 134 diseased. Moreover, in greenhouse trials during the winter of 1930, as in 1931 field trials, the progenies segregated into healthy and diseased classes in a ratio reasonably close to the expected 1 to 1. The total of all trials shows a deviation which might be significant were it not for the explanation already given; in view of the normal behavior of these progenies in the greenhouse trial and in the second field trial, however, the large total deviation is not considered significant.

<sup>6</sup> WALKER, J. C., and SMITH, R. EFFECT OF ENVIRONMENTAL FACTORS UPON THE RESISTANCE OF CABBAGE TO YELLOWS. Jour. Agr. Research 41: 1-15, illus. 1930.

TABLE 5.—Occurrence of yellows in progenies resulting from back crosses between  $F_1$  hybrid plants ( $Rr$ ) (from cross  $BS-3 (RR) \times C-131 (rr)$ ) and plants of a homozygous susceptible cabbage line ( $SP$ ), 1930 and 1931

Cross	Tested in—	Number of plants		Deviation	Probable error	Dev. P. E.
		Healthy	Diseased			
BC-2 $\times$ SP-5.....	{ Field.....	48	29	9.50	$\pm 2.96$	3.21
	{ Greenhouse.....	14	14	0	$\pm 1.78$	0
BC-3 $\times$ SP-5.....	{ Field.....	46	20	13.00	$\pm 2.74$	4.75
	{ do. ".....	28	28	0	$\pm 2.62$	0
BC-4 $\times$ SP-5.....	{ Field.....	42	22	10.00	$\pm 2.70$	3.70
	{ do. ".....	91	96	2.50	$\pm 4.61$	.54
	{ Greenhouse.....	124	121	1.50	$\pm 2.58$	.28
BC-5 $\times$ SP-5.....	{ Field.....	36	23	6.50	$\pm 2.59$	2.51
	{ Greenhouse.....	255	241	7.00	$\pm 7.61$	.93
BC-6 $\times$ SP-5.....	{ Field.....	36	19	8.50	$\pm 2.50$	3.40
	{ Greenhouse.....	34	26	4.00	$\pm 2.61$	1.53
BC-8 $\times$ SP-5.....	{ Field.....	26	27	7.50	$\pm 2.46$	.20
	{ Greenhouse.....	70	55	7.50	$\pm 3.77$	1.99
BC-9 $\times$ SP-5.....	{ Field.....	33	20	6.50	$\pm 2.46$	2.64
	{ Greenhouse.....	100	105	2.50	$\pm 4.83$	.52
BC-10 $\times$ SP-9.....	{ Field.....	29	12	8.50	$\pm 2.16$	3.94
	{ Greenhouse.....	56	68	6.00	$\pm 3.76$	1.60
BC-11 $\times$ SP-9.....	{ Field.....	8	4	2.00	$\pm 1.17$	1.71
	{ do. ".....	28	10	9.00	$\pm 2.08$	4.33
Total in field.....		451	310	70.50	$\pm 9.30$	7.58
Total in greenhouse.....		653	630	11.50	$\pm 12.08$	.95
Grand total.....		1,104	940	82.00	$\pm 15.25$	5.38
Calculated (1:1).....		1,022	1,022			

\* Test made in 1931; other tests were made in 1930.

#### INHERITANCE OF RESISTANCE IN KOHLRABI

Four kohlrabi plants (Kr-2, Kr-3, Kr-4, and Kr-6), selected in the field from the commercial stock, were brought to flower in the greenhouse. Progenies were tested in the field and in the greenhouse in 1929. As shown in Table 6, the progeny of Kr-2 segregated into healthy and diseased classes in approximately a 3 to 1 ratio, although the numbers involved were too low to make this result significant. Kr-3, Kr-4, and Kr-6 appeared to be homozygous for resistance. Certain sib crosses were made involving plants Kr-2, Kr-3, and Kr-4. Trials of the progenies from these crosses showed no yellows, a further indication that Kr-3 and Kr-4 were homozygous for resistance. Moreover, a cross between Kr-3 and susceptible cabbage C-131 yielded a completely resistant progeny. Three plants from the selfed progeny of Kr-3 were grown to seed the following winter. All three were self-pollinated and two were back crossed to susceptible cabbage. All progenies from self-pollination and from back crosses were completely resistant.

TABLE 6.—Occurrence of yellows in progenies from four resistant kohlrabi plants selected from a commercial strain of White Vienna variety, 1929

Progeny from—	Parent plant or cross No.	Tested in—	Number of plants		Genotype class of parent plants
			Healthy	Diseased	
Self-pollination.....	Kr-3.....	{ Field.....	27	0	$RR$ .
		{ Greenhouse.....	29	0	$RR$ .
	Kr-4.....	{ Field.....	27	0	$RR$ .
		{ Greenhouse.....	31	0	$RR$ .
	Kr-6.....	{ Field.....	12	0	$RR$ .
	Kr-2.....	{ do.....	10	4	$Rr$ .
Sib crosses.....	Kr-3 $\times$ 2.....	do.....	28	0	$RR \times Rr$ .
	Kr-3 $\times$ 4.....	do.....	26	0	$RR \times RR$ .
	Kr-4 $\times$ 3.....	do.....	9	0	$RR \times RR$ .
Cross with homozygous susceptible cabbage.	Kr-3 $\times$ C-131.....	do.....	26	0	$RR \times rr$ .

Plants from the hybrid progeny Kr-3×C-131 were saved and brought to flower in the greenhouse. The behavior of the progenies from self-pollination of these plants (KC-1, KC-2, etc.), as tested in the field and in the greenhouse, is shown in Table 7. As in Brussels sprouts, the progenies from self-pollination of the hybrids segregated in a ratio approximating 3 resistant to 1 susceptible. The trials in the field gave somewhat less than the expected number in the diseased class, whereas in the greenhouse trials the reverse was the case.

TABLE 7.—Occurrence of yellows in  $F_2$  progenies from a cross between a homozygous resistant kohlrabi plant (Kr-3) and a homozygous susceptible cabbage plant (C-131)

Progeny No.	Tested in—	Number of plants		Deviation	Probable error	Dev. P. E.
		Healthy	Diseased			
KC-1s.....	Field.....	31	7	2.50	±1.80	1.39
KC-2s.....	do.....	23	6	1.25	±1.57	.80
	do.....	61	11	7.00	±2.48	2.82
KC-4s.....	do. <sup>a</sup> .....	45	27	9.00	±2.48	3.63
	Greenhouse.....	98	42	7.00	±3.46	2.02
KC-5s.....	Field.....	5	2	.25	±.77	.32
KC-6s.....	do.....	20	9	1.75	±1.57	1.11
KC-7s.....	do.....	10	3	.25	±1.06	.24
	do.....	62	13	5.75	±2.53	2.27
KC-8s.....	do. <sup>a</sup> .....	41	21	5.50	±2.30	2.39
	Greenhouse.....	149	53	2.50	±4.15	.60
KC-9s.....	Field.....	22	2	4.00	±1.43	2.80
	Greenhouse.....	11	5	1.00	±1.17	.85
KC-10s.....	Field.....	37	11	1.00	±2.02	.50
	Greenhouse.....	43	15	.50	±2.22	.23
KC-11s.....	Field.....	68	7	11.75	±2.53	4.64
	Greenhouse.....	84	20	6.00	±2.98	2.01
KC-12s.....	Field.....	20	5	1.25	±1.46	.86
	Greenhouse.....	28	12	2.00	±1.85	1.08
KC-13s.....	Field.....	42	8	4.50	±2.07	2.77
	Greenhouse.....	31	15	3.50	±1.98	1.77
	Field.....	55	14	3.25	±2.43	1.34
KC-14s.....	do. <sup>a</sup> .....	48	19	2.25	±2.39	.94
	Greenhouse.....	139	50	9.50	±4.11	2.04
KC-15s.....	Field.....	14	3	1.25	±1.20	.81
KC-16s.....	do.....	37	5	5.50	±1.89	2.91
KC-17s.....	do.....	47	6	7.25	±2.13	3.40
	Greenhouse.....	40	15	1.25	±2.17	.58
KC-18s.....	Field.....	24	4	3.00	±1.55	1.94
	Greenhouse.....	19	13	5.00	±1.65	3.03
Total in field.....		712	183	40.75	±8.74	4.66
Total in greenhouse.....		642	249	26.25	±8.72	3.01
Grand total.....		1,354	432	14.50	±12.34	1.18
Calculated (3:1).....		1,340	446			

<sup>a</sup> Test made in 1931; other tests were made in 1930.

Back crosses of the hybrid plants (Kr-3×C-131) were made with homozygous susceptible cabbage (SP-5 and SP-9). The reaction of the progenies of this type are given in Table 8. The diseased class in the field trial of 1930 contained fewer plants than was expected on the basis of a 1 to 1 ratio, but the discrepancy is explained, as in Brussels sprouts, by the loss of members of the susceptible class in the yellows-infested seed bed. Further trials of three of these progenies were made in the field in 1931. Two segregated in an approximately 1 to 1 ratio; in the third, the number of diseased plants was higher than expected. In greenhouse trials all progenies tested showed approximately the same number in each class.



TABLE 8.—Occurrence of yellows in progenies resulting from back crosses between  $F_1$  hybrid plants ( $Rr$ ) (from cross  $Kr-3$  ( $RR$ )  $\times$   $C-131$  ( $rr$ )) and plants of a homozygous susceptible cabbage line ( $SP$ )

Progeny No.	Tested in—	Number of plants		Deviation	Probable error	Dev. P. E.
		Healthy	Diseased			
KC-1 $\times$ SP-5.....	{ Field.....	35	15	10.00	$\pm 2.38$	4.20
	{ Greenhouse.....	79	87	4.00	$\pm 4.35$	.92
KC-2 $\times$ SP-5.....	{ Field.....	30	16	7.00	$\pm 2.29$	3.06
	{ do. <sup>a</sup> .....	57	53	2.00	$\pm 3.54$	.56
KC-3 $\times$ SP-5.....	{ Greenhouse.....	173	133	20.00	$\pm 5.90$	3.39
	{ Field.....	31	18	6.50	$\pm 2.36$	2.75
KC-4 $\times$ SP-5.....	{ do. <sup>a</sup> .....	29	23	3.00	$\pm 2.43$	1.23
	{ Greenhouse.....	55	82	13.50	$\pm 3.95$	3.42
	{ Field.....	74	64	5.00	$\pm 3.96$	1.26
KC-5 $\times$ SP-5.....	{ do. <sup>a</sup> .....	42	26	8.00	$\pm 2.78$	2.88
	{ Greenhouse.....	33	35	1.00	$\pm 2.78$	.36
	{ Field.....	33	26	3.50	$\pm 2.59$	1.35
KC-6 $\times$ SP-5.....	{ Greenhouse.....	35	17	9.00	$\pm 2.43$	3.70
KC-7 $\times$ SP-5.....	{ Field.....	33	29	2.00	$\pm 2.66$	.75
	{ do. <sup>a</sup> .....	21	15	3.00	$\pm 2.02$	1.49
KC-8 $\times$ SP-5.....	{ Greenhouse.....	30	17	6.50	$\pm 2.31$	2.81
	{ Field.....	30	28	1.00	$\pm 2.67$	.39
KC-9 $\times$ SP-5.....	{ Greenhouse.....	23	19	2.00	$\pm 2.19$	.91
	{ Field.....	26	27	.50	$\pm 2.46$	.20
KC-10 $\times$ SP-5.....	{ Greenhouse.....	29	15	7.00	$\pm 2.24$	3.12
	{ Field.....	30	29	.50	$\pm 2.59$	.19
KC-11 $\times$ SP-5.....	{ Greenhouse.....	32	21	5.50	$\pm 2.46$	2.24
KC-12 $\times$ SP-5.....	{ do. <sup>a</sup> .....	37	16	10.50	$\pm 2.46$	4.27
	{ Greenhouse.....	74	89	7.50	$\pm 4.31$	1.74
KC-13 $\times$ SP-9.....	{ Field.....	30	24	3.00	$\pm 2.48$	1.21
KC-14 $\times$ SP-5.....	{ do. <sup>a</sup> .....	33	29	2.00	$\pm 2.66$	.75
KC-15 $\times$ SP-5.....	{ do. <sup>a</sup> .....	38	18	10.00	$\pm 2.52$	3.97
KC-16 $\times$ SP-9.....	{ do. <sup>a</sup> .....	25	25	0	$\pm 2.38$	0
	{ Greenhouse.....	68	83	7.50	$\pm 4.14$	1.81
KC-17 $\times$ SP-9.....	{ Field.....	19	25	3.00	$\pm 2.24$	1.34
Total in field.....		664	509	77.50	11.55	6.71
Total in greenhouse.....		620	595	12.50	11.76	1.06
Grand total.....		1,284	1,104	90.00	16.48	5.46
Calculated (1:1).....		1,194	1,194			

<sup>a</sup> Test made in 1931; other tests were made in 1930.

## DISCUSSION

The data seem to prove that resistance to the yellows organism is based on a single-factor difference in kohlrabi and Brussels sprouts, as has already been shown to be the case in both cultivated and wild cabbage.<sup>8</sup> Since all these plants belong to the composite species *Brassica oleracea*, it is reasonable to suppose that the same gene is responsible for resistance in all four subspecies. The results herein reported demonstrate the possibility of developing homozygous resistant lines of kohlrabi and Brussels sprouts by selection from individual survivors in commercial stocks grown upon soil thoroughly infested with the yellows organism.

## SUMMARY

It has been shown that when commercial varieties of Brussels sprouts and kohlrabi were planted on yellows-infested soil certain individuals remained free from the disease throughout the growing season. When such individuals were self-pollinated the progenies of some proved to be completely resistant, whereas the progenies of others segregated in a ratio approximating 3 resistant to 1 susceptible.

<sup>8</sup> WALKER, J. C. Op. cit. (See footnote 2.)

When plants from completely resistant progenies were crossed with homozygous susceptible cabbage plants, resistance was found to be completely dominant in the  $F_1$  generation. Progenies from self-pollination of  $F_1$  hybrid plants when grown upon infested soil segregated into resistant and susceptible classes in the ratio of 3 resistant to 1 susceptible. When the  $F_1$  hybrid plants were back crossed with susceptible cabbage plants, segregation of the progenies occurred in the ratio of 1 resistant to 1 susceptible.

These results indicate that, as already shown for both cultivated and wild cabbage, *Fusarium* resistance in Brussels sprouts and kohlrabi is based on a single-factor difference and is probably carried by the same gene in all four subspecies of *Brassica oleracea*.

The present report demonstrates the possibility of developing homozygous resistant lines of Brussels sprouts and kohlrabi by selection from resistant individuals grown on yellows-infested soil.

# EFFECT OF FLUORINE ON THE NUTRITION OF SWINE, WITH SPECIAL REFERENCE TO BONE AND TOOTH COMPOSITION<sup>1</sup>

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## INTRODUCTION

Numerous reports have appeared in the literature purporting to show the value of phosphoritic minerals as calcium and phosphorus supplements in animal nutrition. A great variance exists between the results obtained by different investigators. Forbes and others (9)<sup>2</sup> have inferred that the fluorine content of these naturally occurring products was the cause of the harmful and variable results obtained when these minerals were fed to livestock. Such an assumption seems to be fairly well grounded, since there is some correlation between the fluorine content of various grades of these minerals and the poor results obtained. However, the majority of the investigators have made no attempt to determine whether the feeding of fluorine salts would produce results comparable to those produced when the natural minerals were fed. In the present investigations a study was made of the comparative effects of fluorine, in the forms of chemically pure sodium fluoride and the natural phosphate mineral, in the nutrition of swine, with special reference to the effect on bone and tooth composition.

## REVIEW OF LITERATURE

Different investigators have shown that fluorine in one form or another may prove to be extremely toxic and very often fatal. Baldwin (2) and McNally (24) reported several cases of fluorine poisoning in man. Blaizot (5), Wieland and Kurtzahn (39) and Marcovitch (25) studied the lethal doses of different forms of fluorine for rabbits, while Heidenhain (16) reported on the lethal effects of sodium fluoride in dogs. Using sublethal doses of fluorine in different forms, Schulz and Lamb (30), Bergara (3), Sollmann, Schettler, and Wetzel (32), and McClure and Mitchell (19) observed an impairment of growth and feed consumption in rats.

The effects of fluorine on the various organs and tissues of the body have been studied by numerous investigators. Brandl and Tapeiner (6) could find no histological changes in the blood, liver, kidneys, or muscles of a dog fed varying amounts of sodium fluoride. The same investigators found no evidence of histological change in

<sup>1</sup> Received for publication Jan. 20, 1933; issued July 1933. The data in this paper are taken, in part, from a dissertation by C. H. Kick in partial fulfillment of the requirements for the degree of doctor of philosophy in the Graduate School of the University of Wisconsin.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 1035.

the bones, but reported the presence of crystals—presumably calcium fluoride—in the Haversian canals and in the porous bone near the epiphysis of the long bones. Sollmann, Schettler, and Wetzel (33) reported that they were unable to find histological lesions in rats fed 8 mg of sodium fluoride per kilogram of body weight daily for 9 weeks. Pitotti (27) observed a degeneration of the epithelium of the kidneys and a cloudy swelling of the liver in rabbits and guinea pigs fed lethal doses of sodium fluoride. Siegfried (31) noted that sodium fluoride and sodium fluosilicate caused a destruction of the epithelium of the intestines, even though these salts had been introduced into the body through other channels. Weinland (38) reported that the mucous membrane from the throat of a frog was killed by a 2.1 per cent solution of sodium fluoride. Grützner (13) noted that nerves were equally sensitive. Maumene (26) and Goldemberg (11) each reported that the feeding of sodium fluoride caused an increase in the size of the thyroid glands of dogs and rats, whereas Chaneles (7), and Tolle and Maynard (36) were unable to verify these findings.

The specific effect of fluorine on the teeth of rats has been shown by Schulz and Lamb (30), McCollum, Simmonds, Becker, and Bunting (21), Bergara (3), and Tolle and Maynard (36). The incisors became soft, elongated, and lost their normal pigmentation. Taylor (35), Huffman and Reed (17), and Bohstedt and his coworkers, as reported by Christensen (8) also reported that the teeth of dairy cattle became soft and showed excessive wear when rock phosphate containing fluorine was fed. The latter investigators also reported similar teeth changes in brood sows fed rations containing fairly liberal amounts of rock phosphate. The effect of fluorine ingestion on human teeth has been reported by Smith, Lantz, and Smith (32). These investigators found that children who drank water containing large amounts of fluorine developed soft teeth with mottled enamel. Similar observations were made by the California State Department of Health (1). The only known reference to any change in the chemical composition of the teeth due to fluorine ingestion is that of Sonntag (34), who reported an increase in the fluorine content.

Attempts to study the effect of fluorine feeding on bone formation have for the major part dealt with the use of rock phosphate as a mineral supplement in animal nutrition. Hart and his associates (15) reported improved bone formation when floats (rock phosphate) were used to supplement low phosphorus rations for pigs. Forbes and his coworkers (9), on the contrary, found that the feeding of rock phosphate to pigs produced less dense and weaker bones than when no minerals, or minerals practically free from fluorine, were fed. The weaker bones were characterized by maximum magnesium and phosphorus content and minimum calcium and carbon dioxide percentages. Reed and Huffman (28) demonstrated that the feeding of rock phosphate resulted in a thickening of the jaw and the metatarsal bones of dairy cattle. McClure and Mitchell (19) observed an increase in the ash percentage of bones, accompanied by a slight decrease in the calcium content of the ash, in rats on high fluorine rations. Tolle and Maynard (36), on the contrary, reported a decrease in the percentage of bone ash in rats fed a ration containing 1.8 per cent rock phosphate. The last-named investigators also reported that phosphatic limestone, containing approximately 1 per

cent fluorine, was equal to a mixture of limestone and steamed bone meal for growth and bone formation in the pig.

McClure and Mitchell (20) found that fluorine, in the form of rock phosphate or calcium fluoride, depressed calcium metabolism in the pig and that excessive fluorine intake also decreased growth and feed consumption.

That fluorine is a normal constituent of body tissues has been shown by Zdarek (41), Gautier (10), Wrampelmeyer (40), Jodlbauer (18), Harmes (14), Sonntag (34) and others. Sonntag (34) found that whereas normal teeth and bones of dogs contained not over 0.3 per cent fluorine, the bones of dogs fed sodium fluoride contained 1.73 per cent on the moisture-free and the fat-free basis, and the teeth 1.29 per cent on the dry basis.

#### EXPERIMENTAL PROCEDURE

Two separate experiments were carried out with growing pigs, housed indoors in concrete paved pens. Straw was used as bedding. At the termination of the experiment the animals were slaughtered and subjected to a routine post-mortem examination. The femurs and mandibles were removed for physical and chemical examinations. They were cleaned of adhering flesh and subjected to certain physical measurements, prior to the removal of the teeth for analytical purposes and the crushing and extraction of the femurs for chemical analysis. All the physical measurements and chemical determinations were made on the same femur from each animal in any particular experiment. The kidneys were fixed in 4 per cent formalin and subsequently examined histologically.

The maximum length and smallest diameter of the femurs were obtained by means of vernier micrometers. The volume of the femurs was determined by difference in the weights of the green, cleaned bone in air and when completely immersed in distilled water. Breaking strength determinations were made with an Olsen dynamometer. Calcium and magnesium were determined by McCruden's (22, 23) method, and phosphorus by the official gravimetric procedure. The carbon dioxide in the dry, fat-free bones was determined by the Van Slyke (37) method, and the volatilization method of Reynolds, Ross, and Jacobs (29) was used for the estimation on fluorine.

The same basal rations were used in the two experiments. Previous studies in this laboratory had shown that these rations were satisfactory for growing pigs when properly supplemented with calcium. The starting ration consisted of 55 parts yellow corn, 25 parts wheat middlings, 19 parts linseed meal, 0.5 part sodium chloride, and 0.5 part cod-liver oil. When the animals had attained a weight of approximately 125 pounds, the basal ration of all lots was changed at the same time to one consisting of 75 parts yellow corn, 14 parts wheat middlings, 10 parts linseed meal, 0.5 part sodium chloride, and 0.5 part cod-liver oil. The change in ration was made to widen the nutritive ratio, and conforms to good animal-husbandry procedure. The rations were hand-fed twice daily. The pigs were weighed individually every two weeks.

In the first experiment, which ran for 144 days, eight lots of eight pigs each, weighing at the start approximately 50 pounds each, were used. Four lots of pigs received the basal rations supplemented with 908 g of ground limestone, and 0, 30, 60, and 100 g of sodium fluoride

per 100 pounds of ration. Two more lots were fed the basal rations supplemented with 2 parts of different rock phosphates. Rock phosphate A was of Tennessee origin and analyzed 3.34 per cent fluorine, while rock phosphate B represented a pebble rock phosphate mined in Florida and contained 3.61 per cent fluorine. The pigs in another lot had their ration supplemented with equal parts of ground limestone and rock phosphate A, and the remaining lot received a ration that included 2 parts of steamed bone meal.

The mineral composition of these rations are presented in Table 1. The calcium:phosphorus ratios of the rations of the different lots were variable. However, other work in this laboratory indicates that the variations fall within the range of maximum performance and that the percentages of calcium and phosphorus, or the proportion in which they were present in the ration, were not limiting factors in the growth, or in the bone and tooth formation of these animals.

TABLE 1.—*Mineral composition of rations used for each of eight lots of pigs in Experiment 1*

[Upper figures represent starting ration; lower figures represent finishing ration]

Lot No.	Mineral additions to 100-pound basal ration	Cal-cium	Phos-phorus	Magne-sium	Fluor-ine	Ca:P ratio
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
1	908 g ground limestone.....	0.802 .766	0.527 .422	0.269 .212	(a) (a)	1.5 1.8
2	908 g ground limestone; 30 g sodium fluoride.....	0.802 .766	0.527 .422	0.269 .212	0.029 .029	1.5 1.8
3	908 g ground limestone; 60 g sodium fluoride.....	0.802 .766	0.527 .422	0.269 .212	.058 .058	1.5 1.8
4	908 g ground limestone; 100 g sodium fluoride.....	0.802 .766	0.527 .421	0.268 .211	.097 .097	1.5 1.8
5	454 g ground limestone; 454 g rock phosphate, A.....	.786 .751	.676 .570	.254 .197	.033 .033	1.2 1.3
6	908 g rock phosphate B.....	.772 .736	.827 .721	.236 .180	.070 .070	.9 1.0
7	908 g rock phosphate A.....	.770 .734	.824 .719	.240 .183	.065 .065	.9 1.0
8	908 g steamed bone meal.....	.785 .700	.822 .717	.240 .183	(a) (a)	.9 1.0

<sup>a</sup> Trace.

From the growth and feed-utilization data presented in Table 2, it is obvious that as the percentage of fluorine in the ration increased, the feed intake, the daily gains, and the feed utilization of the animals were materially reduced. These reductions occurred irrespective of whether the fluorine in the ration was derived from rock phosphate or sodium fluoride. The data, in general, substantiate the observations of other investigators that an excess of fluorine in the ration retards growth and feed consumption in various classes of animals.

TABLE 2.—*Gains and feed requirements of various lots of pigs in Experiment 1*

Lot No.	Fluorine in ration	Average initial weight	Average final weight	Average daily gain per pig	Average daily feed per pig	Feed required per 100 pounds of gain
	<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
1.....	(a)	54.6	204.6	1.04	4.92	472.4
2.....	0.029	52.0	192.0	.97	4.48	461.3
3.....	.058	54.0	182.6	.89	4.31	483.1
4.....	.097	52.5	138.0	.59	3.62	610.5
5.....	.033	54.0	196.0	.99	4.84	491.7
6 <sup>b</sup> .....	.079	51.1	139.4	.59	3.89	635.8
7.....	.065	53.1	130.3	.54	3.48	650.1
8.....	(a)	52.1	217.0	1.14	5.02	438.7

<sup>a</sup> Trace.

<sup>b</sup> 1 pig in lot 6 died at 103 days.

Daily observations of the different lots revealed that the pigs in the lots on high-fluorine rations, particularly lots 6 and 7 (2 parts rock phosphate) were consuming considerably more water than were those in the other lots. No attempts were made to record the comparative water consumption of the different lots in this experiment. It was also noted that several of the animals in the fluorine-fed lots manifested signs of stiffness and were rather reluctant to move. This was especially true in the higher fluorine lots. One pig in lot 6 (2 parts rock phosphate B) died at 103 days. Post-mortem examination showed a contracted, roughened, firm, pale kidney.

Gross examination of the femurs showed that those from lots 1 and 8 (2 parts limestone and 2 parts steamed bone meal, respectively) were smooth and dense in appearance, with a normal yellowish color and a definite luster, while those of the other lots were rough, lusterless, white in color, and with some exostoses. These conditions became more pronounced as the amount of fluorine in the ration increased. It is apparent from the data in Table 3 that the breaking strength of the bone is affected by high fluorine intake. Those rations that contained approximately 0.06 per cent of fluorine or more, either in the form of rock phosphate or sodium fluoride, significantly decreased the tensile strength of the femur. The data also indicate that excessive fluorine ingestion increases the diameter of the femur. This is particularly evident when the comparative length and weight of the bone are taken into account. The ash content of the femurs (Table 3) shows some variation. In general, the percentage of ash is lowest in the highest fluorine-fed lots. The difference in ash values are, however, only statistically significant in the case of lots 1 and 4 (control, and 100 g of sodium fluoride, respectively). Further experimental work is required before it can be stated with certainty whether or not excessive fluorine feeding will significantly lower the percentage of bone ash in pigs during short or long feeding periods.

TABLE 3.—Physical measurements and ash analyses of the femurs of pigs in various lots in Experiment 1

Lot No.	Fluorine in ration	Weight	Volume	Length	Small-est diameter	Breaking strength	Ratio of breaking strength to weight of bone	Ash	Grams of ash/cc volume
	Per cent	Grams	Cubic centimeters	Centimeters	Centimeters	Pounds		Per cent	
1.....	(a)	177	135	18.4	2.00	690±62	3.94	61.33±0.39	0.805
2.....	0.029	164	124	17.5	2.06	733±29	4.51	62.34±.27	.825
3.....	.058	167	131	16.8	2.21	647±34	3.92	60.50±.31	.770
4.....	.097	135	111	16.3	2.03	393±19	2.93	58.03±.29	.708
5.....	.033	160	126	17.1	2.12	766±31	4.81	61.86±.51	.760
6.....	.070	133	111	16.3	2.05	407±34	3.21	59.42±.68	.707
7.....	.065	134	109	16.1	2.01	456±40	3.34	60.78±.54	.743
8.....	(a)	177	134	18.3	1.95	841±55	4.80	61.61±.32	.816

\* Trace.

The close correlation between the amount of ash per unit volume of bone and the breaking strength is of special interest. Apparently the ash-volume ratio is a more critical factor in determining the tensile strength of the femur than is the percentage of ash in the dry extracted bone.

The chemical analyses of the bone ash and dry extracted femurs, presented in Table 4, show that increasing percentages of fluorine in the ration had no significant effect on the percentage of calcium and phosphorus in the ash, whereas the percentage of magnesium tended to increase with increased fluorine intake, irrespective of the magnesium content of the ration. The carbonate content of the fat-free bones tended to decrease with increased fluorine intake, while the percentage of fluorine in the bone was directly proportional to the amount ingested.

TABLE 4.—*Chemical analyses<sup>a</sup> (per cent) of the femurs of pigs in various lots in Experiment 1*

Lot No.	Fluorine in ration	Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
1.....	( <sup>b</sup> )	38.39	17.48	0.790	2.76	0.039
2.....	0.029	38.50	17.99	.959	2.72	.534
3.....	.058	38.27	17.68	.972	2.37	.775
4.....	.097	38.43	17.62	1.141	2.34	1.108
5.....	.033	38.10	17.89	1.036	2.57	.594
6.....	.070	37.79	17.91	1.167	1.92	1.037
7.....	.065	37.92	18.14	1.137	2.06	1.093
8.....	( <sup>b</sup> )	38.31	17.75	.823	2.81	.036

<sup>a</sup> The calcium, phosphorus, and magnesium percentages are based on the bone ash; those of the carbon dioxide and fluorine are based on the dry, fat-free bone.

<sup>b</sup> Trace.

The mandibles of the fluorine-fed pigs showed the same thickened condition and rough, white, lusterless appearance that was noted in the femurs. Enlargement of the body of the bone and the appearance of exostoses became more evident with increased fluorine intake. No differences in the amount of wear of the teeth from the various lots were noted. However, there was a tendency for the teeth from the higher fluorine lots to chip more easily than those from the controls. Chemical analysis of the teeth did not show any significant difference in the percentage of ash or in the calcium, phosphorus, and magnesium content of the ash. (Table 5.) The carbon dioxide content of the teeth was not affected, except in lots 6 and 7 (2 parts rock phosphates A and B), which gave evidence of a slight decrease. The percentage of fluorine in the teeth was directly proportional to the amount present in the ration.

TABLE 5.—*Chemical composition<sup>a</sup> (per cent) of the teeth of pigs in various lots in Experiment 1*

Lot No.	Fluorine in ration	Ash	Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
1.....	( <sup>b</sup> )	74.04	35.83	17.48	1.45	2.03	0.083
2.....	0.029	74.06	36.32	17.77	1.54	2.09	.181
3.....	.058	74.17	36.53	17.96	1.63	2.07	.281
4.....	.097	74.69	36.99	17.88	1.53	2.15	.361
5.....	.033	74.84	36.67	17.66	1.51	2.11	.228
6.....	.070	74.44	36.70	17.57	1.50	1.83	.331
7.....	.065	75.17	37.02	17.35	1.56	1.95	.375
8.....	( <sup>b</sup> )	75.80	36.88	17.33	1.79	2.12	.092

<sup>a</sup> Calcium, phosphorus, and magnesium percentages are based on tooth ash; those of carbon dioxide and fluorine on dry, fat-free teeth.

<sup>b</sup> Trace.

Gross examination of the various organs at the time of slaughter revealed no abnormalities, with the exception of the kidneys from the pigs that had been fed rock phosphate. The kidneys were pale in color, contracted, and firm in texture, and their surfaces were



roughened by numerous small nodules and depressions. (Fig. 1, A.) The capsules were slightly thickened, and in some instances firmly adherent to the surface. Occasionally small cysts containing a clear or amber-colored fluid protruded above the surface of, or were more

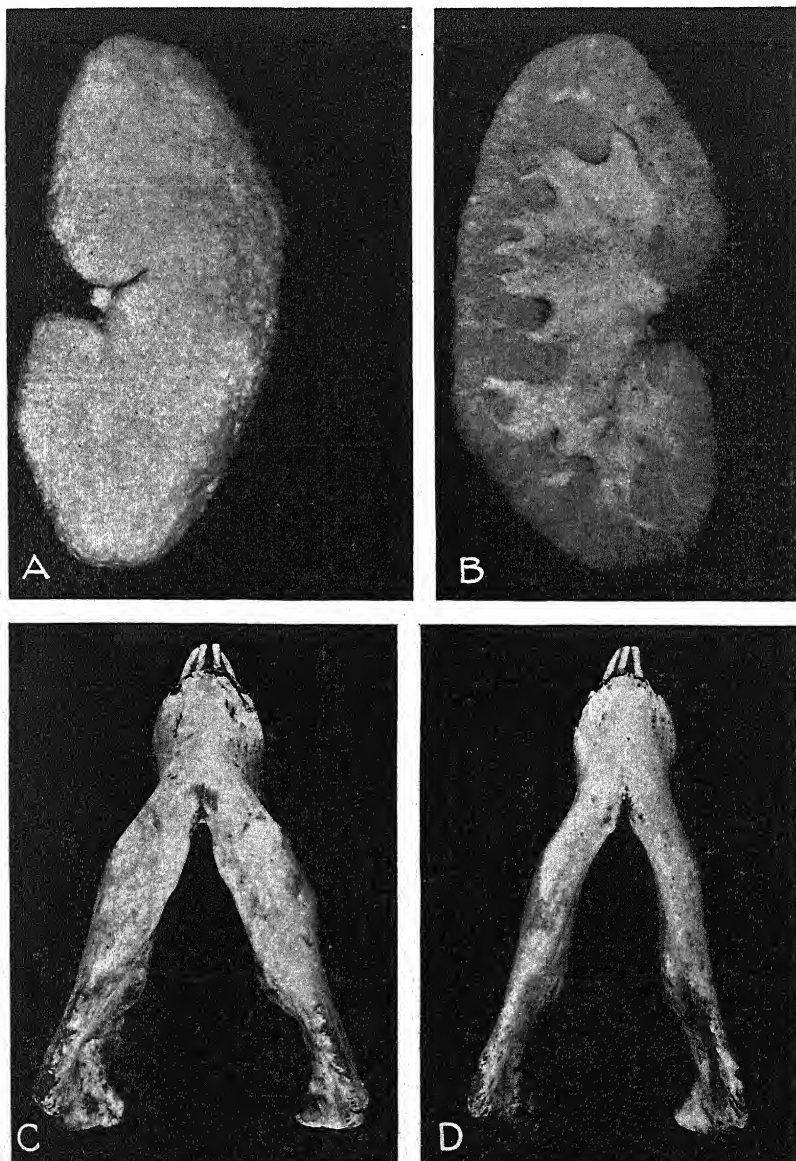


FIGURE 1.—A, External appearance of kidney from a pig that was fed 2 per cent rock phosphate; B, cross section of kidney from a pig that was fed 2 per cent rock phosphate; C, D, mandibles of pigs No. 76 of lot 4 and No. 58 of lot 1, showing increased thickness caused by the ingestion of fluorine

deeply situated in, the kidney. On section the cortex appeared reduced in width, and frequently the medulla contained considerable amount of fat. (Fig. 1, B.)

Microscopically the kidneys showed a nephritis with a varying degree of degeneration of the tubular epithelium and, as a terminal result, the replacement of many tubules and glomeruli with fibrous tissue. (Pl. 1.) None of the animals in the control or sodium fluoride-fed lots exhibited this condition.

In an attempt to confirm the results of the previous trial, and to study the effects of smaller amounts of fluorine in the ration, a second experiment, using six lots of eight pigs each, was started. The basal rations were the same as those used in the first test. The experiment ran for 160 days and was conducted under conditions similar to those in the first experiment. The mineral composition of the rations are presented in Table 6. The amounts of the phosphatic minerals and limestone were so adjusted that the percentages of calcium and phosphorus were the same in the rations of all lots. Fluorine was supplied in varying amounts in the form of sodium fluoride or rock phosphate.

TABLE 6.—Mineral composition of rations used for each of six lots of pigs in Experiment 2

[Upper figures represent starting ration; lower figures finishing ration]

Lot No.	Mineral additions to 100-pound basal ration	Calcium	Phosphorus	Magnesium	Fluorine	Ca:P ratio
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
1	454 g ground limestone; 454 g steamed bone meal.....	0.714	0.654	0.348	(*)	1.1
		.672	.556	.260	(*)	1.2
2	454 g ground limestone; 454 g steamed bone meal; 10 g sodium fluoride.....	.714	.654	.348	0.010	1.1
		.672	.556	.260	.010	1.2
3	454 g ground limestone; 454 g steamed bone meal; 30 g sodium fluoride.....	.714	.654	.348	.029	1.1
		.672	.556	.260	.029	1.2
4	454 g ground limestone; 454 g steamed bone meal; 60 g sodium fluoride.....	.714	.654	.348	.058	1.1
		.672	.556	.260	.058	1.2
5	429 g ground limestone; 227 g steamed bone meal; 222 g rock phosphate.....	.714	.654	.346	.016	1.1
		.671	.557	.258	.016	1.2
6	404 g ground limestone; 444 g rock phosphate.....	.714	.654	.343	.032	1.1
		.672	.557	.255	.032	1.2

\* Trace.

The gains and feed requirements of the different lots are recorded in Table 7. It is evident that when the fluorine content of the ration exceeded 0.029 per cent the rate of gain and the economy of gain were decreased. Smaller amounts of fluorine, either in the form of sodium fluoride or rock phosphate, appeared to be without effect on growth or feed utilization. These observations, in general, substantiate the results of the first trial and other unpublished data of this laboratory.

TABLE 7.—Gains and feed requirements of various lots of pigs in Experiment 2

Lot No.	Fluorine in ration	Average initial weight	Average final weight	Average daily gain per pig	Average daily ration per pig	Feed required per 100 pounds of gain
	<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
1.....	(*)	39.3	243.4	1.28	5.08	398.4
2.....	0.010	37.7	244.6	1.29	4.92	380.2
3.....	.029	38.8	213.6	1.09	4.26	389.9
4.....	.058	39.4	161.3	.76	3.41	450.2
5.....	.016	40.4	246.0	1.29	5.06	394.7
6.....	.032	40.4	219.4	1.12	4.85	434.0

\* Trace.

Again, in this experiment it was observed that the pigs on the high-fluorine rations consumed larger quantities of water. To check this observation the water and feed consumption of all lots were carefully recorded for two weeks. The results (Table 8) show that a high-fluorine intake caused increased water consumption per unit of live weight.

TABLE 8.—*Effect of fluorine intake on water consumption of pigs in various lots in Experiment 2*

Lot No.	Fluorine in ration	Feed consumed	Water consumed	Average weight	Water consumed per 100 pounds of live weight
	<i>Per cent (°)</i>	<i>Pounds</i>	<i>Gallons</i>	<i>Pounds</i>	<i>Gallons</i>
1.....	0.010	945	165	212	77.83
2.....	0.010	964	156	208	75.00
3.....	.029	767	153	188	81.38
4.....	.058	471	159	144	110.41
5.....	.016	927	165	212	77.83
6.....	.032	817	168	190	88.42

<sup>a</sup> Trace.

As in the previous experiment, the femurs of the pigs that had received 0.029 per cent or more of fluorine in their ration were rough, white, and lusterless, whereas the bones from the pigs on lower fluorine rations appeared normal. The data presented in Table 9 again show an increase in the diameter and a reduction in the breaking strength of the femurs of the pigs on the high-fluorine rations. The percentage of ash was not significantly affected by fluorine feeding. However, as in the first experiment, the amount of ash per unit volume of bone was decreased with higher levels of fluorine intake.

TABLE 9.—*Physical measurements and ash analyses of the femurs of pigs in various lots in Experiment 2*

Lot No.	Fluorine in ration	Weight	Volume	Length	Small-est diameter	Breaking strength	Ratio of break-ing strength to weight of bone	Ash	Grams of ash/cc volume
	<i>Per cent (°)</i>	<i>Grams</i>	<i>Cubic centi-meters</i>	<i>Centi-meters</i>	<i>Centi-meters</i>	<i>Pounds</i>		<i>Per cent</i>	
1.....		178	139	18.4	1.96	1,013±38	6.06	61.75±0.34	0.801
2.....	0.010	182	140	18.3	2.04	976±55	5.33	62.85±.42	.826
3.....	.029	168	132	17.3	2.12	871±42	5.25	62.46±.41	.792
4.....	.058	157	131	17.1	2.22	550±23	3.49	60.74±.51	.732
5.....	.016	176	138	18.1	2.03	1,050±58	6.17	62.04±.25	.796
6.....	.032	179	142	18.3	2.10	827±37	4.76	62.62±.30	.792

<sup>a</sup> Trace.

The data on the chemical analysis of the femurs (Table 10) show no significant differences in the calcium and phosphorus contents of the bone ash from the different lots, while the percentage of magnesium increased with increased fluorine intake. Again, the carbonate content of the dry, fat-free femurs was reduced by high-fluorine feeding, whereas the percentage of fluorine in the bone was directly proportional to the amount ingested.

TABLE 10.—*Chemical analyses<sup>a</sup> (per cent) of the femurs of pigs in various lots in Experiment 2*

Lot No.	Fluorine in ration	Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
1.....	( <sup>b</sup> )	37.61	18.52	0.919	2.93	0.057
2.....	0.010	37.60	18.25	.968	3.01	.316
3.....	.029	37.64	18.33	1.001	2.71	.671
4.....	.058	37.74	18.23	1.211	2.31	1.077
5.....	.016	37.72	18.18	1.043	2.88	.409
6.....	.032	38.18	18.95	1.102	2.78	.624

<sup>a</sup> Calcium, phosphorus, and magnesium percentages are based on bone ash; those of carbon dioxide and fluorine on the dry, fat-free bone.

<sup>b</sup> Trace.

Gross examination of the mandibles revealed the same rough, white, lusterless condition in the high-fluorine lots, as noted in the previous trial. No difference could be detected in the amount of wear of the teeth from the various lots, although it was observed that the teeth of the high-fluorine lots had a tendency to chip more easily than those from the control or low-fluorine lots. The rami of the mandibles were increased in diameter as a result of high-fluorine intake. (Fig. 1, C, D.) This enlargement of the mandibles also caused an increase in the width of the dental arch at the point of the second molar. It was also found that the buccolingual dimensions, taken at the positions of the three molars, increased in direct proportion to the amount of fluorine in the ration. The vertical dimensions, taken at the same points, showed no significant differences.

The analysis of the teeth (Table 11) showed no significant difference in the percentage of ash or carbon dioxide on the dry, fat-free basis. Neither did the tooth ash show any variation in the amount of calcium, phosphorus, or magnesium. The only variable noted was in the fluorine content of the dry, fat-free teeth. The amount present was directly correlated with the percentage in the ration.

TABLE 11.—*Chemical composition<sup>a</sup> (per cent) of the teeth of pigs in various lots in Experiment 2*

Lot No.	Fluorine in ration	Ash	Calcium	Phosphorus	Magnesium	Carbon dioxide	Fluorine
1.....	( <sup>b</sup> )	73.49	36.74	17.86	1.65	1.76	0.043
2.....	0.010	74.39	36.99	17.84	1.67	1.66	.127
3.....	.029	73.96	37.05	17.65	1.60	1.76	.262
4.....	.058	73.93	36.69	17.71	1.66	1.76	.373
5.....	.016	74.60	36.77	17.70	1.70	1.64	.124
6.....	.032	74.21	36.82	17.06	1.74	1.65	.228

<sup>a</sup> Calcium, phosphorus, and magnesium percentages are based on tooth ash; those of carbon dioxide and fluorine on dry, fat-free teeth.

<sup>b</sup> Trace.

A post-mortem examination revealed no abnormalities in the various organs, with the exception of the kidneys of the pigs from lot 6 (approximately 1 per cent rock phosphate). These organs showed the nephritis noted in the animals in the previous trial. None of the kidneys of the pigs from the other lots showed any pathological change.

In a third experiment pigs were fed rock phosphate at levels of 1 and 2 per cent of their ration and sodium fluoride at levels of 30 and

60 g per 100 pounds of ration for two years or more, extending through the complete reproduction cycle. Upon post-mortem these pigs showed bone and kidney changes similar to, but more extensive than, those observed in the experiments reported herein. The condition of, and changes in, the teeth of these long-time fluorine-fed pigs were much more marked than those of pigs in the short-time experiments. The detailed findings of the tooth changes have been reported elsewhere (4). Suffice it to state that frequently the teeth of the high-fluorine-fed pigs were worn to the gums, and the pulp cavities were exposed. This condition of the teeth was reflected in the manner in which the pigs consumed their feed. Upon taking a mouthful of feed the pigs would throw their heads backward in what appeared to be an attempt to swallow the feed without allowing it to come in contact with the molar teeth. No noticeable effort was made to chew the feed. The same animals were reluctant to drink cold water. It was also found that sows reared and maintained on high-fluorine rations did not consume sufficient feed for good lactation or to prevent them from becoming unthrifty.

#### DISCUSSION

From the results of these experiments, it is evident that excessive fluorine in the ration exerts definite physiological effects upon swine. These effects, in general, were the same whether the fluorine was derived from rock phosphate or sodium fluoride. The only difference noted was the effect that liberal amounts of rock phosphate (1 per cent or more of the ration) exerted on the kidneys. Equivalent or even higher levels of fluorine, in the form of sodium fluoride, had no such effect. Two explanations present themselves as to the cause of this discrepancy. Either the naturally occurring product must contain some substance or material, other than fluorine, which causes a pathological change in the kidney, or the fluorine in rock phosphate is present in a more toxic form than in sodium fluoride.

The increased water consumption of the pigs on the high-fluorine levels may in part be accounted for by the results of Gottlieb and Grant (12). These investigators recently reported that the intravenous injection of sodium fluoride into dogs caused a marked diuresis. Although no urinary volume determinations were attempted in the present experiments, it was noted that the litter in the pens of the high-fluorine-fed pigs was much more soiled with urine than that in the other pens. A marked increase in urine volume must therefore be accompanied by an increased water intake in order to avoid a serious dehydration of the body tissues.

The outstanding effect of fluorine on the femurs of pigs, noted in these investigations, was the decrease in breaking strength of these bones. Although the bones of the animals which received the larger amounts of fluorine were larger in diameter and contained approximately the same percentage of ash, their tensile strength was significantly less than bones from those in the low-fluorine lots. Obviously, the breaking strength of a bone is influenced by factors other than ash content. The close correlation between the amount of ash per unit volume of bone and its breaking strength suggest that the ash to volume ratio is a better criterion of the tensile strength of a bone than is the percentage of ash. The fact that the weakest bones possessed the greatest diameter and contained the least ash per unit

of volume, suggests that fluorine may cause some change in the cellular structure of the bones.

The results of the effects of fluorine ingestion on the inorganic composition of the femurs substantiate the findings of Forbes and his associates (9), that the weak bones caused by rock-phosphate feeding are characterized by maximum magnesium and minimum carbon dioxide percentages, but are in disagreement with the report of the same investigators that the proportions of calcium and phosphorus are also changed. It is of interest that the high magnesium and low carbonate percentages were independent of the magnesium or carbonate contents of the rations, the source of calcium and phosphorus in the rations, and the form in which fluorine was ingested. This shows that excessive fluorine intake alters the inorganic composition of the bone. Whether this change in composition as a result of excessive fluorine ingestion is due, in part, to a partial replacement of the carbonate by the fluoride, or an actual shift in the percentages of the different bone salts normally present to compensate for the increased fluorine content, requires further detailed study.

The mandibles of pigs which had been fed toxic amounts of fluorine showed changes similar to those described by Huffman and Reed (17) in the case of dairy cattle fed rock phosphate. On cross section, it was observed that the marrow cavities were greatly enlarged without noticeable increase in the thickness of the bone wall. A marked difference was also noted between the types of bone marrow in the high-fluorine and the low-fluorine lots. The marrow of the low-fluorine lots was red, while that from the high-fluorine lots was pale yellow and appeared to have a decided increase in fatty material. The difference in the size of the marrow cavity and color and texture of the marrow was particularly evident in animals that were fed sodium fluoride or rock phosphate for an extended period.

It was evident that fluorine exerted a definite effect on tooth structure in swine. This fact was especially noticeable in animals fed fluorine for two or more years. These tooth changes were similar to those reported by Huffman and Reed (17) in the case of dairy cattle fed rock phosphate. The chemical analysis of the teeth did not reveal any striking differences in their inorganic composition, aside from the increase in the percentage of fluorine, the increase being directly correlated with the fluorine content of the ration, which substantiates the work of Sonntag (34) with dogs.

The results are in agreement with the work of other investigators with different species of animals, in that excessive fluorine in the ration decreases growth and feed consumption, and the efficiency with which the feed is utilized. The data, in general, show that when more than approximately 0.03 per cent fluorine is included in the ration the normal performance of the pig is retarded. This is especially true in case of long-continued fluorine feeding. Under these conditions, the feed consumption is impaired to such an extent in sows suckling pigs that lactation is adversely affected, and frequently the animals become so unthrifty that it is impossible to bring them back to good condition on the same feed.

It should be pointed out that the average daily calcium and phosphorus intake of the pigs in the different lots in the same experiment were not always uniform, for the reason that the percentages of these elements in the rations between lots varied, or the daily feed intake

between lots was not the same. The authors are of the opinion that these variations in calcium and phosphorus intake do not detract from the significance of the results obtained, in that the minimum requirements for these elements were amply satisfied in all instances. Forbes and his associates (9) and McClure and Mitchell (20) obtained good results with pigs on a daily intake of 5 to 7 g of calcium. In the experiments herein reported, the daily calcium intake was from 2 to 3 times that fed by the above-mentioned investigators, and in all probability the requirements of the pigs for calcium and phosphorus were not limiting factors in the results secured.

#### SUMMARY

The feeding to pigs of rations containing approximately 0.03 per cent or more of fluorine derived from rock phosphate or sodium fluoride impaired growth and feed consumption and lessened the efficiency with which the rations were utilized. The femurs of the pigs were characterized by an increase in the diameter of the shaft, a loss of normal color and luster, the presence of exostoses, and a decreased breaking strength. These changes became more pronounced as the fluorine content of the ration increased.

The bones weakened by fluorine feeding contained normal percentages of ash, calcium, and phosphorus, increased amounts of magnesium and fluorine, and decreased percentages of carbon dioxide. The increased magnesium and fluorine contents and the decreased carbonate content were directly correlated with the percentage of fluorine in the ration. The feeding of fluorine caused an increase in the size of the rami of the mandibles. This enlargement was due to an increase in the size of the marrow cavity rather than to an increase in the thickness of the wall. The type of marrow in the cavity was also changed.

Excessive amounts of fluorine in the ration when fed over long periods softened the teeth of pigs. The percentages of ash, calcium, phosphorus, magnesium, and carbon dioxide in the teeth were not significantly affected by fluorine feeding; however, the fluorine content was directly proportional to the amount fed.

The fluorine from rock phosphate and sodium fluoride exerted the same physiological effects on swine, with the exception that rock phosphate caused certain pathological changes in the kidneys. A nephritis with resulting induration of the kidney occurred in varying degrees in all pigs fed approximately 1 per cent or more of rock phosphate in their ration.

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1. The first part of the paper discusses the importance of the study of the history of the United States. It is argued that the study of the history of the United States is essential for a full understanding of the country and its people. The paper then discusses the importance of the study of the history of the United States in the context of the world. It is argued that the study of the history of the United States is essential for a full understanding of the world and its people. The paper then discusses the importance of the study of the history of the United States in the context of the future. It is argued that the study of the history of the United States is essential for a full understanding of the future and its people.

# VITAMIN C CONTENT OF BALDWIN APPLES AND APPLE PRODUCTS<sup>1</sup>

By C. R. FELLERS, *Research Professor*, M. M. CLEVELAND, *Fellow*, and J. A. CLAGUE, *Research Assistant*, *Department of Horticultural Manufactures, Massachusetts Agricultural Experiment Station*

## INTRODUCTION

In spite of the importance of apple as a food, only a few investigations of its vitamin C content have been reported. A study of the literature failed to reveal any data bearing on the important Baldwin variety. Nor have apple cider and other apple products been carefully evaluated for vitamin content. Although Bracewell, Hoyle, and Zilva (1)<sup>2</sup> found that the Bramley Seedling variety grown in England protected 300-g guinea pigs when fed at a daily level of 3 g, Van Leersum and Hoogenboom (9), Givens, McClugage, and Van Horne (6, 7), Kohman, Eddy, and Carlsson (8), Hessler and Williams (reported by Campbell (4, p. 68)), and Hessler and Craig (reported by Campbell, (3, p. 67-68)) found that the protective amounts of various varieties of apples varied from 10 to over 40 g. At the present time the apple is not generally considered a particularly good source of vitamin C.

This paper presents the first results of a study now being made of the important apple varieties of the Northeast and the effect of various factors upon the vitamin content of apples and of products manufactured from apples.

## VITAMIN C IN SPRAYED AND UNSPRAYED APPLES

In 1932, Nelson and Mottern (10) reported that lead arsenate sprays applied to orange trees considerably reduced the vitamin C content of the fruit. Since apples are almost universally sprayed with arsenic compounds and other toxic substances for insect and fungus control, comparisons were made in the present investigation between sprayed and unsprayed Baldwin apples. The apples for the experiment were obtained from orchards at Haydenville, Mass., and from the college orchard at Amherst, Mass. The usual spray schedule was followed, lime-sulphur and lead arsenate being used. The college orchard received eight and the Haydenville orchard seven sprays. The season was dry, hence there was a considerable spray deposit on the sprayed fruit when it was picked. From 0.005 to 0.008 grain of  $As_2O_3$  per pound of fresh fruit was found on the sprayed fruit, but the unsprayed showed merely a trace of arsenic. The sprayed fruit was a deeper red and of better quality than the unsprayed. However, only sound fruits were used in the feeding experiments. The apples were stored at 36° F. until the test was concluded. Examination was begun in October, 1931, immediately after harvest.

<sup>1</sup> Received for publication Nov. 7, 1932; issued July 1933. Contribution No. 153 of the Massachusetts Agricultural Experiment Station. Presented before the Division of Agricultural and Food Chemistry at the eighty-fourth meeting of the American Chemical Society, Denver, Colo., Aug. 24, 1932.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 1045.

The quantitative animal feeding method of Sherman, La Mer, and Campbell (11) was followed, except that the guinea pigs were fed in proportion to their weight; that is, if an animal weighing 300 g received 3 g of apple daily, one weighing 400 g would receive 4 g of apple. This method is believed to be fairer than feeding the same weight of food regardless of the animal's weight. At least three animals housed in individual wire cages, were used for every test. Radial sectors of apples were fed because of the variation in vitamin C content in different parts of the apple (2, 5).

The basal ration consisted of 58 per cent equal parts of rolled oats and wheat bran, 30 per cent of vitamin-C-free baked milk powder,

10 per cent butterfat, 1 per cent each of cod-liver oil and salt. This basal ration and water were kept before the animals at all times. However, the guinea pigs were forced to derive all their vitamin C from apples or apple products.

At the end of the feeding period all animals were chloroformed and carefully examined for lesions of scurvy. Negative controls died in from 26 to 33 days with an average Sherman scurvy score of 16. A normal animal has a score of 0, while the maximum for extremely severe scurvy is 24.

The significant data are presented graphically in Figure

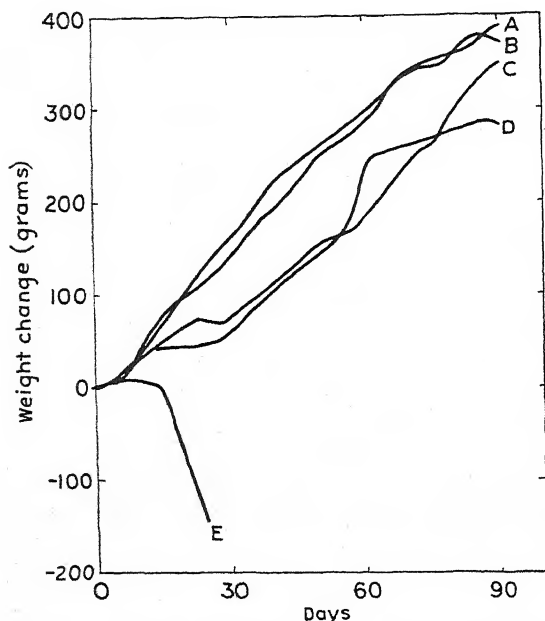


FIGURE 1.—Results of feeding guinea pigs sprayed and unsprayed Baldwin apples from Haydenville and Amherst, Mass., as the sole source of vitamin C: A, 4 g sprayed apple (Haydenville) fed daily, scurvy score, 0; B, 5 g sprayed apple (Amherst) fed daily, scurvy score, 0; C, 5 g unsprayed apple (Amherst) fed daily, scurvy score, 0; D, 4 g unsprayed apple (Haydenville) fed daily, scurvy score, 0; E, negative control, scurvy score, 10

1. Apples from trees carefully sprayed all season are fully as rich in vitamin C as apples from unsprayed trees. In fact, the animals receiving sprayed fruit showed slightly larger weight gains than those receiving the unsprayed. Four grams daily of either sprayed or unsprayed Baldwin apples per 300-g guinea pig gave excellent weight gains and full protection from scurvy.

Two additional series of animals were fed 7 g of sprayed and unsprayed Baldwin apples from Amherst. The average weight gains per animal after 90 days were 381 and 430 g respectively, and there was no evidence of scurvy at autopsy. The data thus show no decrease of vitamin C in the sprayed fruit. In fact in two of the three tests the sprayed fruit appeared to contain slightly more vitamin C than the unsprayed.

### VITAMIN C IN APPLES STORED FOR DIFFERENT LENGTHS OF TIME

Sprayed Baldwin apples grown on the college farm and stored at 36° F. were used to determine the effect of storage on vitamin C. The examinations for vitamin C were made in October immediately following harvest, after 3 to 5 months in storage, and finally after 8 to 10 months. Figure 2 shows the results obtained.

Four grams of fresh apple possessed approximately the same anti-scorbutic potency as 5 g of 4-months-old apple or 6 g of 9-months-old fruit. Similarly, 3 g of apple in storage 4 months gave better growth and protection than 4 g after 9 months. After 9 months the loss in vitamin C was about 33 per cent. Baldwin apples apparently retain a substantial part of their vitamin C for several months; that is, during the season in which they are eaten in largest quantities. Bracewell, Kidd, West, and Zilva (2), and also Van Leersum and Hoogenboom (9) found no loss in vitamin C of apples during storage, but Hessler and Craig, as reported by Campbell (3), found a loss of fully 30 per cent in Jonathan apples held in storage about 5 months.

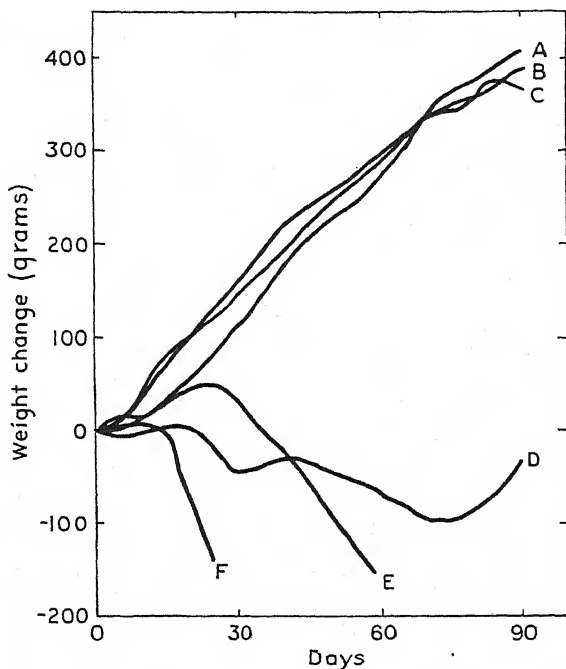


FIGURE 2.—Effect of storage on the vitamin C content of Baldwin apples as shown by the results of feeding them to guinea pigs as the sole source of vitamin C: A, 6 g apple stored 9 months fed daily, scurvy score, 0; B, 4 g fresh apple fed daily, scurvy score, 0; C, 5 g apple stored 4 months fed daily, scurvy score, 0; D, 3 g apple stored 4 months fed daily, scurvy score, 5; E 4 g apple, stored 9 months fed daily, scurvy score 13; F, negative control, scurvy score, 10

### VITAMIN C IN APPLE JUICE AND PRESERVED CIDER

Apple juice was extracted daily from sprayed Baldwin apples which had been in storage 9 months and whose protective dose was approximately 6 g. The small centrifugal extractor shown in Figure 3 was used to extract the juice. The yield of juice averaged 70 to 80 per cent of the weight of the apple. The juice was pale green in color when first extracted but oxidized to a deep yellowish brown after 24 hours.

By the use of a hydraulic press cider was prepared from the apples that had been in storage at 36° F. for 4 months. Guinea pigs fed 5 g daily of these apples were fully protected from scurvy. The cider was divided into two portions. One was heated to 170° F. and allowed

to settle at 36° for 12 hours, siphoned into pint bottles, sealed with crown caps, and pasteurized for 20 minutes at 160°; the other was benzoated by adding sodium benzoate sufficient to give a concentration in the cider of 0.1 per cent. Both lots were stored in 1-pint bottles at 36°. It was necessary to make up a second batch of benzoated cider after the experiment had been in progress 40 days. The effect

of feeding this freshly prepared cider is evident by the marked rise in Figure 5, C, at the 40 to 45 day interval. All juices and cider were fed by pipette, so that each animal received a known amount each day. (Fig. 4.)

The significant data are presented in Figure 5.

The animals receiving 9 g (not shown in fig. 5) and 6 g daily of freshly extracted apple juice were well protected against scurvy and showed large gains in weight. Three grams of the fresh juice, however, did not afford protection. Since 6 g of the whole apples afforded protection, it appears that freshly extracted juice is practically as rich in vitamin C as the apple itself. Nine grams of 1-day-old juice also afforded full



FIGURE 3.—Small centrifugal extractor used in preparing the apple juice

protection, showing that the juice retains a substantial part of its vitamin C for at least 24 hours. Lesser amounts were not evaluated.

Pressed cider differs from extracted cider in both appearance and flavor. There is greater opportunity for oxidation to take place in the pressed product. The benzoated cider possesses moderate antiscorbutic properties for several days after manufacture but gradually loses this property on storage. Eight grams fed daily failed to support growth or give protection from scurvy. Probably 10 to 15 g would have been required. As pointed out previously, the temporary upward trend after 40 days in Figure 5 indicates increased growth as a result of feeding freshly prepared cider. No animal fed 4 g daily survived for more than 60 days.

Pasteurized cider when fed at the 8-g level showed practically no protective value and was little better than the negative controls. There is a marked loss in vitamin C when Baldwin apples are manufactured into benzoated or pasteurized cider. Unless the juice is freshly extracted from apples, it is likely to contain but little vitamin C.

#### VITAMIN C IN CANNED APPLE SAUCE

The vitamin C content of two kinds of Baldwin apple sauce was determined. Unstrained, "lumpy" or rough sauce, was prepared from peeled and cored fruit. The waste was 23 per cent. Sugar equivalent to 18.7 per cent of the weight of prepared apples and water equivalent to 13 per cent was added and the sauce was cooked in a loosely covered kettle for 6 minutes. Since the yield was exactly the weight of the prepared apples, 10 g of the finished sauce was practi-

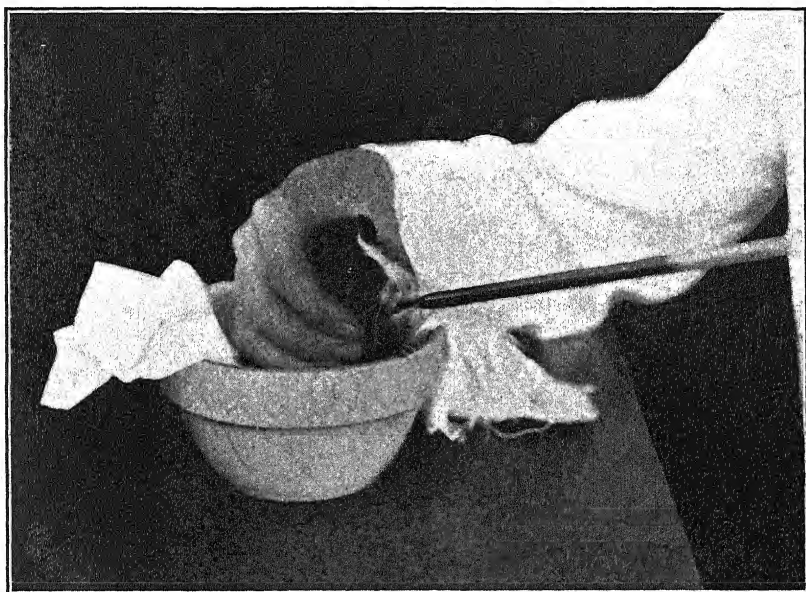


FIGURE 4.—Pipette method of feeding apple juice and cider to guinea pigs

cally equivalent to 8 g of apple. The sauce was packed into half-pint jars, pasteurized at 170° F. for 20 minutes, and immediately cooled.

The smooth or strained apple sauce was prepared by cooking quartered unpeeled Baldwin apples until soft. Twelve per cent by weight of water was added before cooking. The mass was pulped while hot in a tomato pulper, 19 per cent sugar was added, and the sauce was packed hot into half-pint glass jars and pasteurized at 170° F. for 25 minutes. The sauce was stored at 36° until used, a new jar being opened every other day. Most of the guinea pigs ate the sauce greedily; those that did not were eliminated from the experiment. The graphs representing the collected data are shown in Figure 6.

In general, canned apple sauce does not retain much of the vitamin C of the fresh apple. Strained sauce, even when fed at 10-g levels

was little better than the negative control. Certainly the quantity of strained apple sauce necessary to protect against scurvy would be

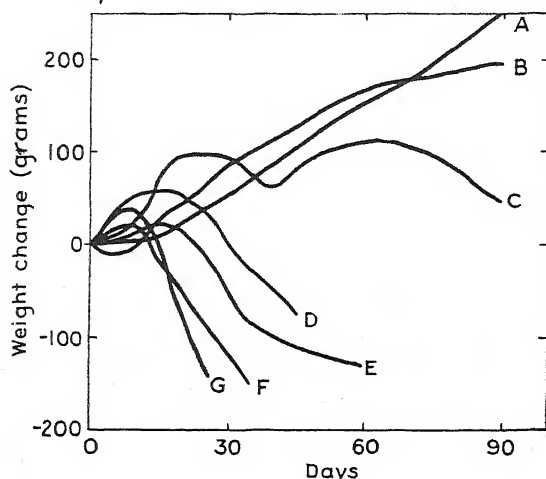


FIGURE 5.—Results of feeding guinea pigs Baldwin apple juice and apple cider as the sole source of vitamin C: A, 9 g 1-day-old apple juice fed daily, scurvy score, 0; B, 6 g fresh apple juice fed daily, scurvy score, 1.5; C, 8 g of benzoated cider fed daily, scurvy score, 10; D, 3 g fresh apple juice fed daily, scurvy score, 10; E, 4 g benzoated cider fed daily, scurvy score, 14; F, 8 g pasteurized cider fed daily, scurvy score, 14; G, negative control, scurvy score, 10

very high. The unstrained sauce was somewhat richer in vitamin C, but as much as 10 g was not nearly enough to protect the animals against scurvy or to maintain their weight. Neither style of sauce, as prepared in this experiment, can be considered of appreciable antiscorbutic value. These results are in accord with those of Hessler and Williams (4), who found that Johnathan apple sauce contained practically no vitamin C, and with those of Koh-

man, Eddy, and Carlsson (8) who obtained similar results with Yellow Newtown (Albamarle Pippin) and Stayman Winesap.

### SUMMARY

The Baldwin apple grown in Massachusetts is a good source of vitamin C, about 4 g per 300 g of body weight sufficing to maintain growth in guinea pigs and to protect them fully from scurvy.

In two tests, apples from completely sprayed trees contained as much or possibly more vitamin C than apples from unsprayed trees.

In 4 to 6 months of storage at 36° F. Baldwin apples lost about 20 per cent of their vitamin C content; in 8 to 10 months the loss was nearly 40 per cent.

Freshly expressed Baldwin apple juice was found to be nearly as rich in vitamin C as the fresh apple. Little loss occurred during the

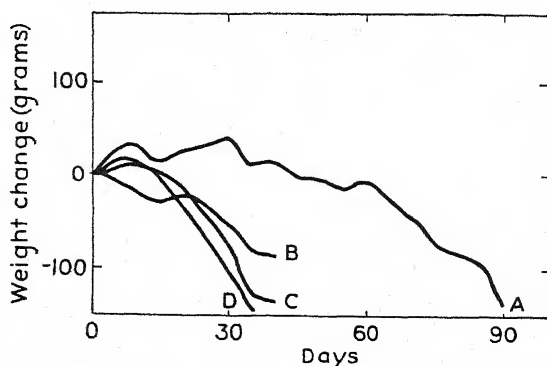


FIGURE 6.—Results of feeding guinea pigs Baldwin apple sauce as the sole source of vitamin C: A, 10 g unstrained apple sauce fed daily, scurvy score, 9; B, 5 g unstrained apple sauce fed daily, scurvy score, 13; C, 10 g strained apple sauce fed daily, scurvy score, 14; D, 5 g strained apple sauce fed daily, scurvy score, 14



first 24 hours after extraction. Benzoated or pasteurized ciders over 48 hours old, however, failed to retain an appreciable quantity of vitamin C.

Canned Baldwin apple sauce, either strained or unstrained, proved to be a poor source of vitamin C. The unstrained sauce was somewhat superior to the strained.

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# SOMATIC SEGREGATION IN A SECTORIAL CHIMERA OF THE BARTLETT PEAR<sup>1</sup>

By V. R. GARDNER, *Director*, J. W. CRIST, *Research Assistant Horticulturist*, and R. E. GIBSON, *Michigan Agricultural Experiment Station*

## INTRODUCTION

Mention is made in some of the older European pomologies of a considerable number of striped, banded or "panache" varieties or strains of pears. Apparently they never assumed much commercial importance and even academic interest in them must have largely disappeared, for more recent treatises have been inclined to overlook them and a search to-day would probably reveal the fact that most of them have disappeared from cultivation. Descriptions vary in their completeness, but it is evident that the fruits themselves were generally characterized by alternating sectors of the normal green and of yellow skin, extending from stem to calyx lobes, and that at least in many instances the bark of the shoots was similarly striped. Historical data pertaining to many of these sports are likewise incomplete, but the presumption is that most, if not all, originated as bud sports from normal solid green or self-colored varieties. Undoubtedly they would be now classified as sectorial chimeras.

In the summer of 1912 one of the authors of this article, in company with Dr. E. J. Kraus, then of the Oregon Agricultural Experiment Station, found a limb sport of this type in a tree of the Bartlett variety, near Medford, Oreg. Buds of this limb sport, set on quince stock on the grounds of the Oregon station at Corvallis, produced striped fruit like that borne by the original limb sport. No note was made at the time the original limb sport was found as to whether more or less than half of the surface of the fruits or of the shoots was yellow, or on the color distribution of either fruits or shoots as grown at the Oregon station. The sport was simply observed and propagated as a striped form. In 1923 scions obtained from the Oregon station trees were top grafted on some young Kieffer pear trees growing in the orchard of the Michigan experiment station, and the resulting grafts (four on one tree and two on another) have in turn furnished stock for more recent propagations. A study of the Michigan-grown stock of this strain has furnished the data relating to bud-sport origin reported in this paper.

## DESCRIPTION OF MATERIAL

This striped bud sport of the Bartlett pear is apparently fairly representative of bud sports of similar type listed in the older pomologies. The developing fruits show alternating vertical stripes of yellow and green, the individual stripes being of various widths.

<sup>1</sup> Received for publication Nov. 14, 1932; issued July, 1933. Journal Article No. 127 (n. s.) from the Michigan Agricultural Experiment Station.

(Fig. 1.) In some specimens the green predominates, in others the yellow. For the most part each stripe is continuous from base to apex of fruit, though many are broken, being green at the basal end and yellow at the apical end, or vice versa. This striping is clearly evident when the developing fruits are less than a quarter of an inch in diameter. As the fruits approach maturity, the stripes become less distinct because of the gradual disappearance of the green pigment. The fruit stems are not striped but are very much lighter in color than those of normal Bartletts, being distinctly yellowish in cast.

The young shoots of this sport similarly show an alternate striping with green and yellow; this condition likewise characterizes the new growth of the spurs. Toward the end of the growing season as the epidermis cracks and weathers and the corky layer of the cortex

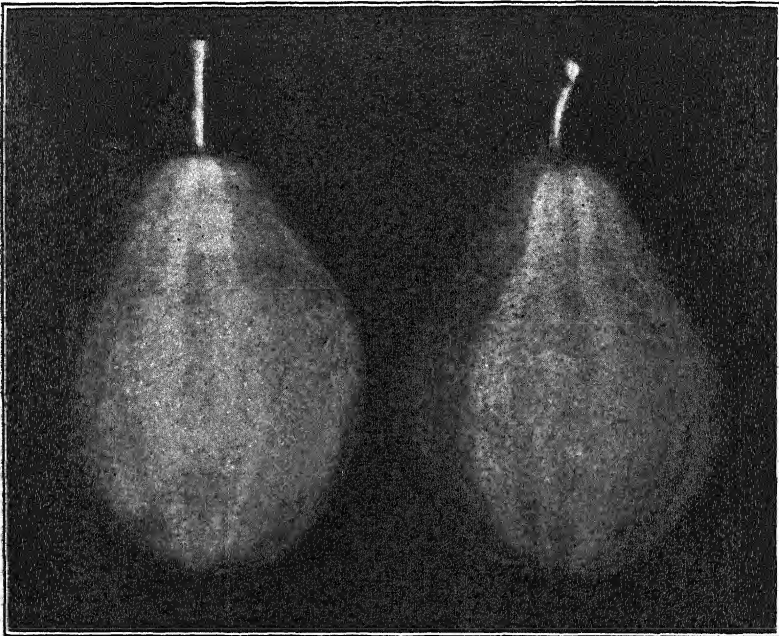


FIGURE 1.—Typical specimens of the striped Bartlett, fully grown but still firm, and before the normal green color changes to yellow

becomes exposed, the striping disappears and the year-old shoots can not be readily told from those of a normal Bartlett. The leaves of the bud sport show no visible evidence of variegation and appear to be as large and as green as those of the normal unstriped form.

In a general way both fruits and shoots of the top grafts in the orchard of the Michigan station closely resemble those of the original limb on the parent tree near Medford, Oreg.

#### EXPERIMENTAL PROCEDURE

##### COLOR STRIPING OF SHOOTS

After the top grafts at the Michigan station had made two or three years' growth, it was noted that certain of the striped shoots on tree 1 were predominantly yellow, their green stripes being reduced to narrow

vertical lines and constituting not more than 10 per cent of the surface of the shoots; other shoots were predominantly green, their yellow stripes being similarly reduced to narrow bands, but constituting perhaps one-fourth of the total surface. Buds were selected from several of these shoots showing different proportions of the two color stripes and were set in seedlings pear stocks to determine whether it is possible to segregate from this striped strain other strains or sub-strains distinct in the relative amounts of green and yellow pigment in their bark.

Nursery trees grown from buds cut from striped shoots in which the green tissue predominated grew satisfactorily, though more slowly than adjoining trees of the normal Bartlett. Those grown from buds cut from striped shoots in which the yellow predominated made a very slow growth and at the end of the first season in the nursery were little over half the size of normal stock. (Fig. 2.) At the end of the second year in the nursery the differences were still more pronounced, the trees from the predominantly yellow buds stocks being very small, much-branched and having the general appearance of inferior seedlings, though they had received the best of care. Any nurseryman would classify them as culls, and it is doubtful if any experienced propagator or pomologist would recognize them as being of the Bartlett variety.

The best of the 2-year-old nursery trees of these predominantly yellow selections were set out in the orchard in the spring of 1928,

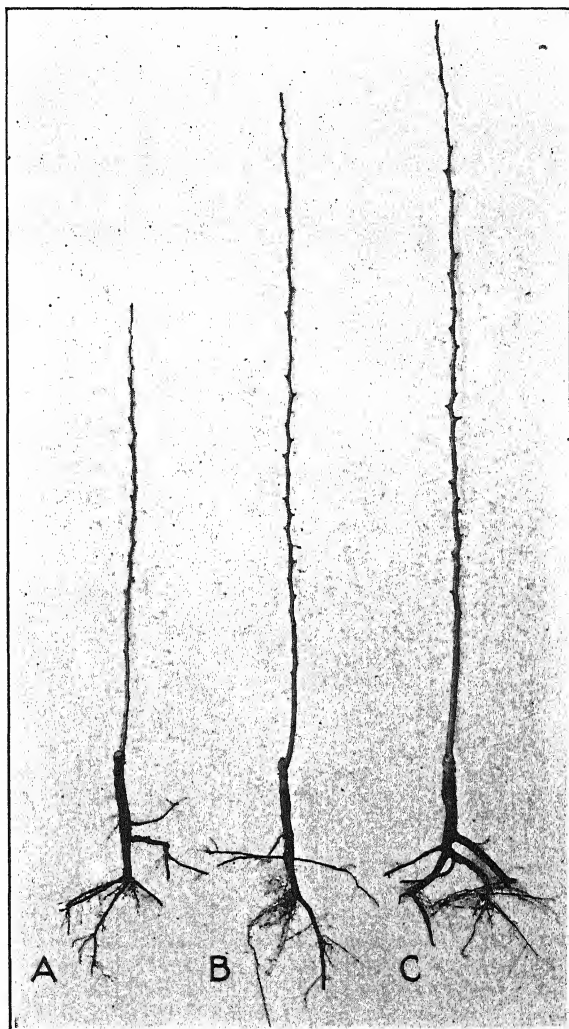


FIGURE 2.—Typical 1-year-old nursery trees of Bartlett selection: A, Striped form in which yellow color predominates; B, striped form in which green color predominates; C, normal trees with solid green bark. Note differences in size

along with some of those of the striped but predominantly green type, and some of the normal Bartlett for comparison. Several of those of the predominantly yellow type died; those that lived have made a comparatively weak growth. Figure 3 shows a representative tree of each selection after two years' growth in the orchard. After five years' growth in the orchard the difference in size between these groups of trees, though still great, was less pronounced than at the end of the first two years.

The marked differences in vegetative growth between these two striped strains and between them and the normal Bartlett, even

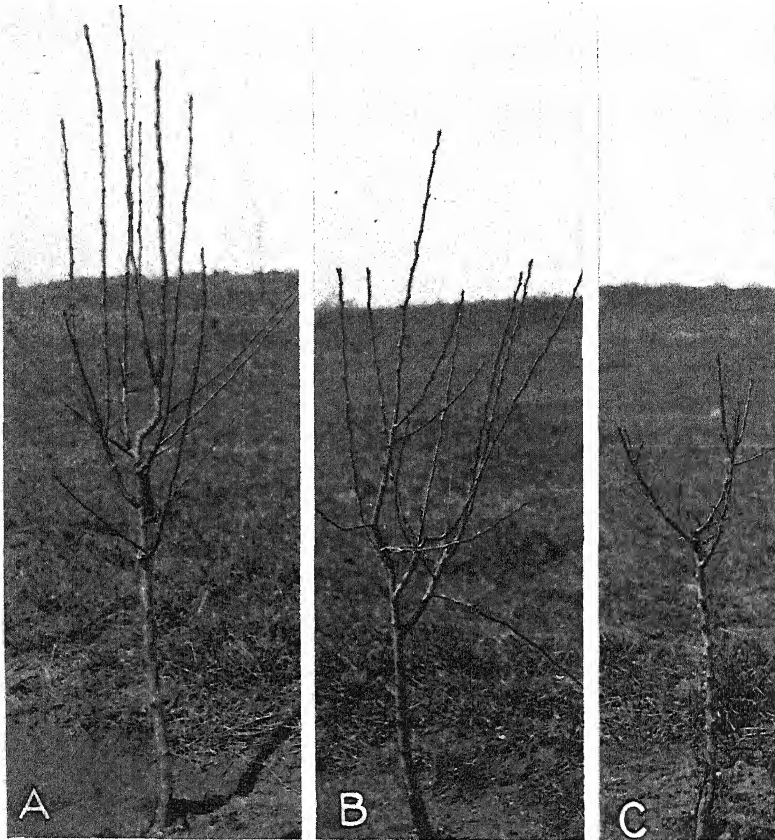


FIGURE 3.—Typical 2-year-old orchard tree of Bartlett selection: A, A normal all-green tree; B, a striped form with the green predominating; C, a striped form with the yellow predominating

though there were no apparent differences in size, thickness, or color of leaves, suggested the desirability of chlorophyll determinations on both leaves and bark of the shoots. Such determinations were made on young nursery stock of the three strains, samples being taken July 11 and August 13, 1932. The data are presented in Table 1. It is clear that, even though to all appearances the leaves of the two striped forms were as dark green as those of the check, or normal Bartlett, they actually contained distinctly less chlorophyll. The bark of the

striped strain in which the yellow color predominated was very low in the green chlorophyll pigments, and it likewise contained much less xanthophyll and carotin than did the bark of the normal Bartlett shoots. The bark of the striped shoots in which the green color predominated showed an intermediate condition. Incidentally these analyses, together with the growth records of the nursery and young orchard trees, suggest that the bark is a more important contributing factor in general vegetative growth and development than has been commonly thought.

TABLE 1.—*Chlorophyll and yellow pigment content of leaves and bark of shoots of normal and striped Bartlett strains*

[Expressed in terms of milligrams per 10 g of fresh material]

Date and strain	Chlorophyll (A+B)		Xanthophyll		Carotin		Total yellow pigments	
	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
July 11, 1932:								
Normal solid green Bartlett.....	15.02	4.44	1.27	0.21	1.18	0.24	2.45	0.45
Striped, with green predominating.....	9.66	3.27	1.18	.15	.77	.26	1.95	.41
Striped, with yellow predominating.....	9.63	-----	1.16	.02	.95	.03	2.11	.05
Aug. 13, 1932:								
Normal solid green Bartlett.....	25.51	3.68	1.92	.20	.90	.15	2.82	.35
Striped, with green predominating.....	21.39	2.94	1.49	.11	.91	.09	2.40	.20
Striped, with yellow predominating.....	18.54	2.65	1.77	.09	.96	.06	2.73	.15

TABLE 2.—*Production of shoots and fruits of different color types by striped Bartlett grafts in 1932*

Color type of shoots and fruits	Number of shoots and fruits by tree and branch indicated					
	Tree 1, branch A	Tree 1, branch B	Tree 1, branch C	Tree 1, branch D	Tree 2, branch A	Trees 2, branch B
Shoots:						
Predominantly yellow.....	13	25	35	19	29	0
Predominantly green.....	5	7	3	3	4	1
Solid green.....	3	0	1	<sup>a</sup> 6	7	0
Fruits:						
Predominantly yellow.....	52	30	10	150	34	27
Predominantly green.....	22	7	8	95	41	87
Solid green.....	3	0	0	<sup>b</sup> 32	0	3

<sup>a</sup> Including shoots produced by the lower branch that had reverted to the normal Bartlett type.

<sup>b</sup> Including fruits produced by the lower branch that had reverted to the normal Bartlett type.

The success that attended the segregation of the original striped Bartlett into two striped color strains led to a careful scrutiny of all the shoots on the two trees in the orchard at East Lansing in the summer of 1932 to determine whether or not further segregation was taking place. The results of the observations are presented in Table 2. Most of the shoots were striped, but on four of the original six grafts one or more all-green shoots were produced, that is, in these cases there had been a complete reversion to normal. Of the 144 striped shoots on these 6 top grafts, 121 were classified as having yellow predomi-

nating, 23 as having green predominating. There seems to be no definite proportion of pure green or of principally green segregates characterizing all the grafts. One summer shoot (fig. 4) had its growth stopped in early June and then produced three secondary lateral branches, one of which was pure green, the second striped with green predominating, and the third striped with yellow predominating. Here is a case of both partial and complete color segregation in branches arising from successive nodes on the same shoot. An even more interesting case of color segregation, this one taking place in a single shoot, is illustrated in Figure 5. In this instance the entire

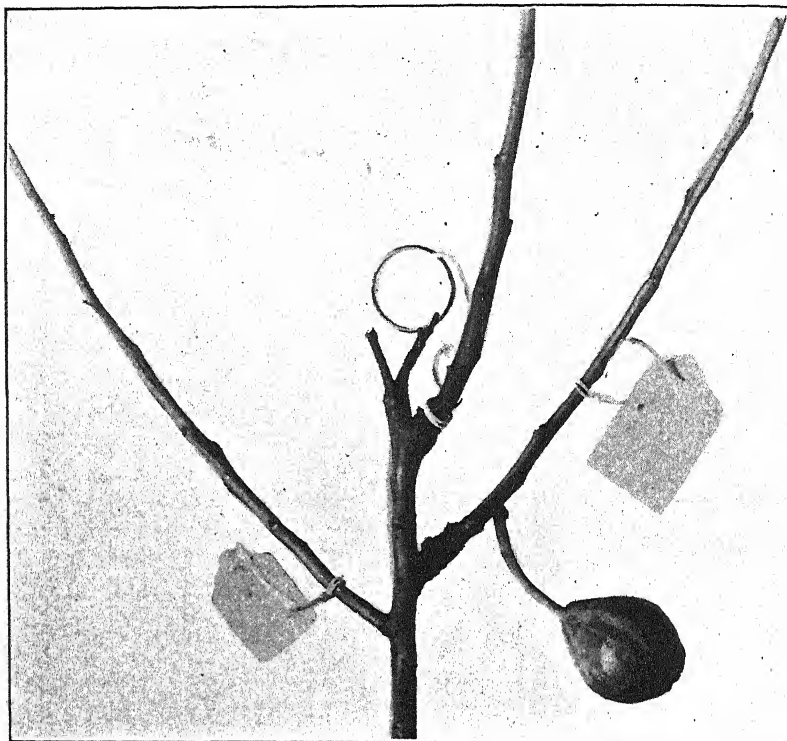


FIGURE 4.—A striped shoot of the striped Bartlett that had its growth stopped in midsummer and then gave rise to three lateral branches. The one at the left is striped but predominantly green; the one at the right is likewise striped but predominantly yellow; the one in the center is the normal solid green

shoot was striped; its basal third was predominantly yellow, the green stripes being reduced to mere pencillike lines; its median section was predominantly green, the yellow stripes being reduced to mere lines; the apical third was predominantly yellow, like the basal third. A close inspection of Figure 5 shows that these color changes occur at the nodes. A vertical green stripe that traverses a number of nodes and internodes may be replaced by a vertical yellow stripe that traverses the next one or more internodes, or vice versa; or a broad green and narrow yellow stripe that traverses one or more internodes may be replaced by a narrow green and broad yellow stripe at some node, or vice versa. One other shoot was found (besides that in fig. 5) in which there was a similar change from internode to internode in the



relative proportions of green and yellow, though none was found in which one or more internodes of an otherwise striped shoot were pure green or in which one or more internodes of an otherwise pure green shoot were striped.

The 1932 records of shoots of these six top grafts would therefore seem to indicate that this particular striped strain is, at least from a vegetative standpoint, in a more or less inconstant or ever-sporting condition and raise a question as to whether it can ever be fixed or segregated into constant substrains. Fortunately some light is thrown on these questions by the progeny trees propagated from two of these six top grafts. Altogether, several hundred nursery trees have been grown from these selections, and a number have been planted in the orchard and carried along until they are now 5 years of age. All trees thus far grown from buds cut from shoots that were striped, but with the yellow stripes predominating, have produced shoots that almost without exception have been like the parent shoots. Those that have grown from buds cut from shoots that were striped, but with the green stripes predominating, have for the most part produced shoots that similarly were like the parent bud sticks, but there have been a few reversions to the pure green normal Bartlett form, and there has likewise been some segregation of striped and predominantly

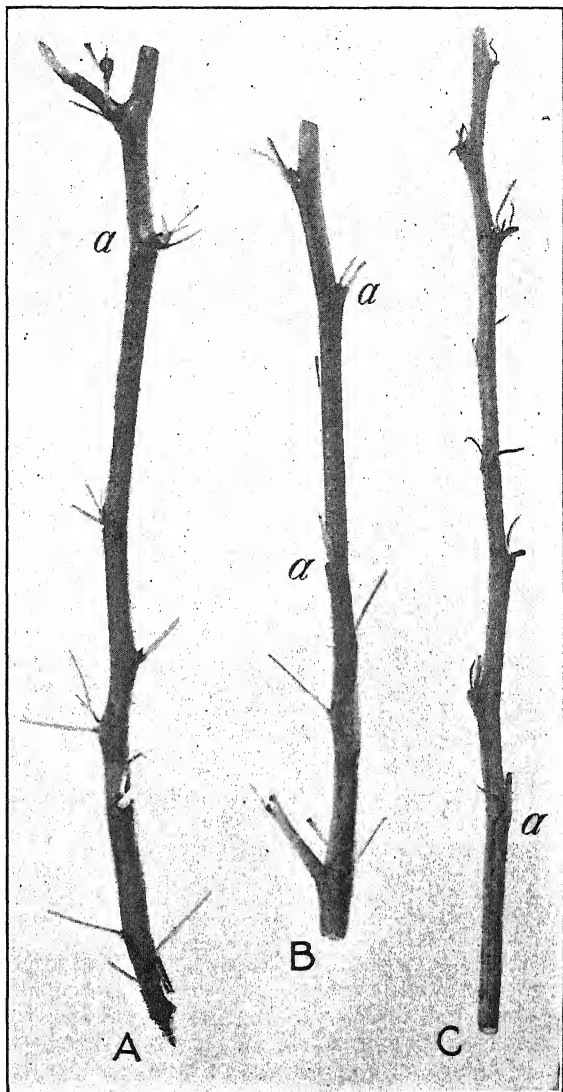


FIGURE 5.—Basal (A), median (B), and subterminal (C) sections of a single shoot of the striped Bartlett in which the basal section is predominantly yellow, the median section predominantly green, and the subterminal section predominantly yellow. Note the color changes taking place at certain of the nodes (a), a green stripe traversing one or several internodes being replaced by a yellow stripe, or vice versa.

have been a few reversions to the pure green normal Bartlett form, and there has likewise been some segregation of striped and predominantly

yellow types. The parent form, as found in Oregon, plainly was not constant or fixed. The subtypes (except for the reversion to the normal all-green Bartlett type) that have segregated from it are likewise not entirely fixed; but they are nevertheless much more constant, and experience with them indicates that they can be kept reasonably constant by suitable care in the selection of bud wood—and only in that way.

#### COLOR STRIPING OF FRUITS

Year after year it has been noted that the fruits themselves of the striped sport vary considerably in width of the alternating yellow and green sectors and in the percentages of the surface covered by the two colors. Not until 1932 were detailed records made relating to this color distribution. They are summarized in Table 2. Considering the crop as a whole, there were many more of the fruits in which the yellow predominated, just as in the shoots, though there was no uniformity in percentages between the different branches. The fruits borne by successive spurs on the same year's growth were in some cases all of one type, and in others presented all possible combinations. In some instances where two fruits were borne by one spur they were alike in color markings, in others one was predominantly green while the other was predominantly yellow. In one case one of a pair coming from a single flower cluster was striped and the other was solid green, a complete reversion to the normal parent Bartlett form. Apparently the successive nodes of the fruit-cluster base present the same opportunity for variation in the width of sectorial color stripes as is presented by successive nodes of the normal vegetative shoot. One whole branch (from graft 1D) that developed from the basal bud of the original scion showed complete reversion to the normal Bartlett type, producing solid green shoots and solid green fruits only, while the main growth of this graft, coming from the upper bud of the scion, has been producing the characteristically striped fruits. Evidently this one branch, in its origin and subsequent behavior, is comparable to one of those shown in Figure 4, and the parent scion for graft 1D corresponded closely to the upper 4 inches of the primary shoot shown in Figure 4.

#### SHAPE OF FRUIT

The Bartlett pear, like certain other pear varieties, presents considerable variation in shape of fruit, though, so far as the writers are aware, evidence has not been presented showing that the variety presents definite dimorphism or polymorphism in this respect. However, close examination of the fruits borne by the six top grafts of its striped sport in the experiment station orchard at East Lansing would suggest the existence of a dimorphic or possibly polymorphic condition. (Fig. 6.) It was therefore thought advisable in 1932 to keep separate the crops as harvested from the six grafts, measure each fruit, and calculate its form index. Table 3 shows the frequency distribution of these form indices (obtained by dividing the total axial by the total transverse diameter, each measured to the closest eighth of an inch). What the writers would consider an ideally shaped, typical, or normal Bartlett has a form index of approximately 1.25 to 1.35, that is, its axial diameter is about a quarter to a third

greater than its transverse diameter. A typical Kieffer pear has a form index of approximately 1.00. It will be noted that two of these striped Bartlett grafts (2A and 2B) produce fruits more or less closely approaching the typical Kieffer shape, their mean form indices being 1.07 and 1.03, respectively. Grafts 1A, 1B, 1C, and 1D bear fruits showing wider ranges in form than do the fruits of grafts 2A and 2B, but many of them are typical Bartletts in form and their mean form indices are much closer to the 1.25 to 1.35 form index than they are to the 1.00 form index of Kieffer. These differences in shape can not be attributed to stock influence because all the scions were top worked on the one stock (Kieffer).

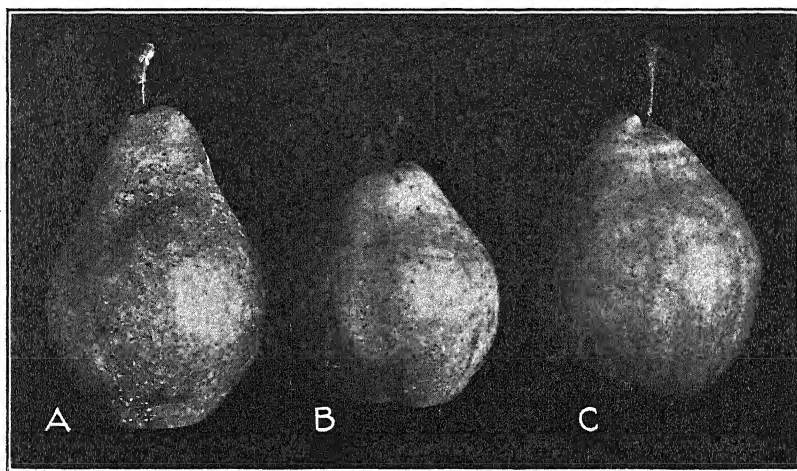


FIGURE 6.—Normal (A) and "Kieffer-shaped" (B and C) specimens of the striped Bartlett. These particular fruits show only indistinct stripes because of their advanced degree of maturity

TABLE 3.—Frequency distribution of the form indices of fruits borne by different striped Bartlett grafts

[Form index determined by dividing the axial diameter of the fruit by its transverse diameter, both having been measured to the nearest eighth of an inch]

Form-index class	Number of pears in class borne by tree or branch indicated										
	1-A	1-A-1	1-A-2	1-A-3	1-A-4	1-A-5	1-B	1-C	1-D	2-A	2-B
0.93 to 0.97									3	7	8
0.98 to 1.02	4	1		1	2				13	19	44
1.03 to 1.07	15	5		2	8			1	57	42	42
1.08 to 1.12	20	10		1	8	1	2	3	49	29	22
1.13 to 1.17	20	16			4		18	9	92	17	3
1.18 to 1.22	6	4			1	1	13	9	90	6	1
1.23 to 1.27	12	7	1		4		22	7	50		
1.28 to 1.32	3	2			1		5	6	11		
1.33 to 1.37	3	1	1		1		7	3	4		
1.38 to 1.42							3		1		
1.43 to 1.47							1				
1.48 to 1.52											
1.53 to 1.56								1			
Total	83						72	39	370	120	123
Weighted average of form indices	1.15						1.24	1.23	1.15	1.07	1.04

Before harvesting it was noticed that the fruits borne by graft 1A not only seemed somewhat less typically Bartlett in form than those of grafts 1B, 1C, and 1D, but also that several of the individual branches of this graft differed rather markedly from each other. Consequently they were harvested separately. The form indices of the fruits borne by these different lateral branches appear in separate columns in Table 3. Obviously, the numbers are not great enough to warrant attaching much significance, and another year's or several years' records must be obtained and propagation tests resorted to before conclusions can be drawn. It may be pointed out, nevertheless, that the same tendency toward dimorphism or polymorphism, toward segregating out into fairly distinct shape types, is evident in the lateral branches of graft 1A as is evident when grafts 1A, 1B, 1C, and 1D are compared with grafts 2A and 2B.

It is recognized that the forms that are here called form segregates are probably incomplete segregates, just like the first color selections. Especially is this true of the so-called normal Bartlett form that is found to some extent in grafts 1A, 1B, 1C, and 1D.

Field and laboratory studies now in progress indicate that many normal Bartlett trees, that is, those producing the normal green fruits, now growing in commercial orchards present this same diversity in fruit shape and that more or less segregation of the type here described is taking place.

No correlation was found between degree or amount of color striping and segregation as to form, some of the predominantly yellow fruits being Kieffer shaped, others typically Bartlett shaped. Similarly, some of the fruits that were predominantly green were Kieffer shaped, while others were of the normal Bartlett form. The evidence, therefore, indicates that out of the one parent limb sport at least four strains have been segregated—two in color and two in shape. The color segregates were the result of artificial selection; the form segregates were purely accidental.

## DISCUSSION

Though the data here recorded pertain to a type of variation that in itself is of little importance in fruits, they throw light on what seems to be a fundamental, though little recognized, characteristic of many bud variations in fruits. This particular variation obviously did not appear as a departure from type at once permanent and fixed, ready to propagate true indefinitely as a uniform new strain or variety (uniform in the sense of meeting the usual specifications of a vegetatively propagated variety). It was a new strain, but a variable, segregating strain from which several fairly definite concrete forms could be developed. This was possible only by what has generally been termed bud selection, and furthermore the evidence indicates that these forms, once developed, can be maintained as clear-cut entities only by a continuation of the same process. This is not surprising in view of what has come to be common practice among experienced propagators of certain ornamental plants such as *Pandanus veitchi*. Incidentally the facts presented raise the question as to whether or not, if more or less continuous selection is necessary in maintaining the identity of certain bud sports, is it not likewise necessary in maintaining the identity of some of the parent varieties from which they may be derived?

## SUMMARY

There is described a sectorial chimera of the Bartlett pear from which has been segregated two color forms and two forms differing from each other in shape of fruit.

The bark of the young shoots of the predominantly yellow segregate contains much less and its leaves contain somewhat less chlorophyll than corresponding tissues of the predominantly green segregate or of the parent all-green form.

Segregation as to color of bark and skin of fruit is independent of segregation as to shape of fruit.

The evidence indicates that continued selection is necessary properly to maintain, as well as to isolate, the types resulting from segregation.



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## MORPHOLOGY OF REPRODUCTION IN CERATOSTOMELLA FIMBRIATA<sup>1</sup>

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### INTRODUCTION

The following report of the results of investigations on a species of the genus *Ceratostomella* is offered primarily to reveal a process of ascus multiplication that appears to be entirely unique in the field of mycology. This process, while doubtless confined to a relatively small number of the Pyrenomycetes, is regarded as an ordinal character and is presented here as a preliminary to further work in this group. To discuss the subject properly, it is desirable to review the grosser morphological characters of the fungus and to trace the sexual stage from the beginning of perithecial development to the discharge of mature ascospores.

The genus *Ceratostomella* is grouped with the Sphaeriales of the Pyrenomycetes and includes both parasitic and saprophytic forms. It is well represented among wood-staining fungi of forest trees and lumber yards. *Ceratostomella fimbriata* (Ell. and Hals.) Elliott is important economically as the cause of black rot of sweetpotatoes (*Ipomoea batatas* (L.) Lam.) (16).<sup>3</sup> The disease was first described by Halsted (12) in 1890, who gave to the fungus the name *Ceratocystis fimbriatum* Ell. and Hals., and the following year Halsted and Fairchild (13) published a more thorough description of the life history of the organism.

Owing to the early disintegration of ascus walls, the perithecia were thought to be pycnidia of an imperfect fungus. Saccardo (23), still regarding the fruiting body as a pycnidium, gave the first technical description of the organism and transferred it to the form genus *Sphaeronema*. The first report that 8-spored asci are formed in the perithecia was made by Elliott (6) in 1923, and accordingly *Sphaeronema fimbriatum* (Ell. and Hals.) Sacc. became *Ceratostomella fimbriata* (Ell. and Hals.) Elliott. In a later paper attributed to Elliott (7) and published after his death, an attempt was made to trace in detail the development of perithecia and asci.

These students, attempting to fit the morphology of *Ceratostomella* into that of a conventional Ascomycete, did not discover the real character of the sexual stage. An examination of the perithecial contents reveals indeed that there are here features never adequately

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<sup>2</sup> Acknowledgment is made to Dr. R. F. Griggs, professor of botany at the George Washington University, for valuable constructive criticism during the progress of the investigation.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 1077.

accounted for in any treatise on Ascomycete morphology known to the present writers. The need for a reexamination of the development of the black-rot organism has led to the present investigation.

The following observations on the life history of *Ceratostomella fimbriata* were made from material grown on corn-meal and potato-dextrose agars. The endoconidia and early stages in perithecial development were observed satisfactorily in water mounts of live mycelium, but the greater part of the study was made from material fixed in Bouin's picric acid solution, sectioned 4-5 $\mu$  in thickness, and stained with Flemming's triple combination of safranin, gentian violet, and orange G. The characteristic structures were shown almost equally well in material fixed in Carnoy's and stained with Heidenhain's haematoxylin.

### ASEXUAL REPRODUCTION

The process whereby the conidia of *Ceratostomella fimbriata* are abstricted within the sheath of the conidiophore has been discussed at some length by Halsted and Fairchild (13), and more recently Lehman (20) has contributed some additional facts. The asexual spores of *C. fimbriata* are of two types, oval spores of olive-brown pigmentation and hyaline spores that are mostly linear in shape and extremely variable in size.

Lehman (20) presented evidence to show that the walls of the endoconidia were generated anew by the protoplast and were not the result of longitudinal splitting of the conidiophore wall as claimed by Brierley (2) for the endoconidia of *Thielavia basicola* (B. and Br.) Zopf. Additional evidence of this fact is adduced here from the frequent presence of an intercalary element between newly formed endoconidia. The structure shown at *a* in Figure 1, G, may consist of lamellar substance that has adhered to the base of the newly abstricted spore. Lehman (20) further contended that the thick-walled olive conidia were in no case formed endosporously, since the protoplast is distended from the ruptured tip before the conidium is abstricted. (Fig. 1, C, *a*.) In material with olive conidia formed in great abundance, a considerable number of spores can be found with two distinct walls, the inner wall appearing to be that of an endospore. (Fig. 1, B.) In Figure 1, A, the outer wall of the spore is shown to be continuous with the sheath of the conidiophore, with the line of rupture just visible.

Germination of the thick-walled olive conidia (fig. 1, D), figured by Harter and Weimer (16), appears to occur rarely. Hyaline conidia (fig. 1, E to L) are capable of germinating as soon as they are discharged from the sporophore, and water mounts from any area of a colony on agar media will usually show large numbers of germinated and germinating spores which disintegrate after having formed a

### EXPLANATORY LEGEND FOR FIGURE 1

- A-D.—Olive-brown thick-walled conidia.  $\times 1,500$ . A and B indicate that the first abstricted spore is double walled; spores produced later from the same conidiophore are single walled.  
 E-L.—Hyaline conidia.  
 E.—Conidiophore showing hyphal fusion at its base.  $\times 1,000$ .  
 F.—Separation of the spore wall from the conidiophore end wall at *a* as a result of plasmolysis.  $\times 1,500$ .  
 G.—Probably a remnant of the middle lamella (*a*) shown at base of recently abstricted spore.  $\times 1,000$ .  
 H, I.—Germinated spores forming new endoconidia.  $\times 1,000$ .  
 J.—Early stage in germination.  $\times 1,000$ .  
 K, L.—Evacuation of spore contents after germination; contrast with H.  $\times 1,000$ .  
 A-D and F from stained sections, others from live material.



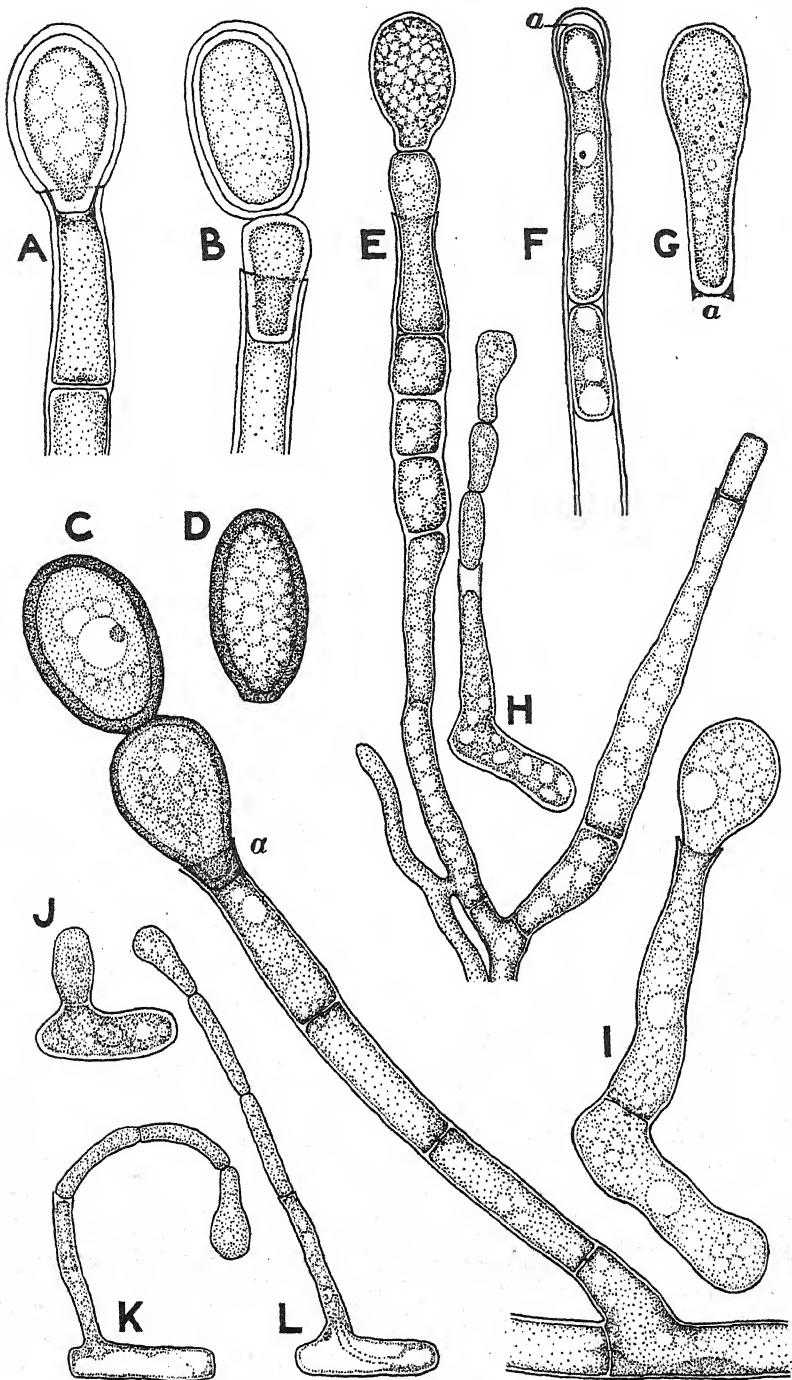


FIGURE 1.—Endoconidia of *Ceratostomella fimbriata*  
For explanatory legend see opposite page

chain of smaller endoconidia. There is normally no evacuation of spore contents as appears in Figure 1, K, L. The one or more germ tubes form at a characteristic angle to the conidium (fig. 1, H to L), and may proceed at once to the formation of new endoconidia (fig. 1, H).

In Petri-dish cultures and on sweetpotato roots in moist chambers, the aerial chains of conidia collapse and become suspended in droplets of water which condense among the aerial hyphae. Under these conditions large numbers of the hyaline spores germinate and the germ tubes grow downward to the surface of the medium. Conidia discharged beneath the surface of the agar germinate and grow upward, discharging new endoconidia above the surface of the medium. Germinating conidiospores very frequently fuse with one another and with neighboring hyphae, forming an irregular entanglement of aerial mycelium having considerable rigidity.

#### INITIATION AND DEVELOPMENT OF PERITHECIA

Mature perithecia of *Ceratostomella fimbriata* are black, membranaceous, and appear dark brown under magnification. The long beak or perithecial neck may reach a length of 1 mm. (Fig. 2, A.) Perithecia frequently occur on the host under field and storage conditions but are formed more abundantly on agar plate cultures. They are normally superficial, sometimes partially immersed, and frequently inclosed in an almost stromatic growth of conidiophores and germinating conidia. (Fig. 2, B.) Perithecia are formed more abundantly in certain cultures than in others, but a large number of single ascospore isolations have failed to reveal any evidence of sexual strains. The antheridium and oogonium are borne on the same hyphal branch. Two or more perithecium initials are occasionally involved in what later develops into a single perithecium (fig. 3, P), but such a coalescence of two or more perithecia may occur at any later stage in development and has no sexual significance. (Pl. 1, A.) Many types of hyphal fusions and fusions of conidia with mycelium occur in all cultures and are regarded as having no relation to sex. The fungus is, therefore, considered to be completely self-fertile, or perhaps parthenogenetic, since the antheridium does not appear to function in fertilization.

The perithecium initial is earliest recognized as a hyphal branch with a thin-walled and slightly recurved hyaline tip and with one or more short hyaline lateral branches. (Fig. 3, A to O.) The perithecial stalks appear to be identical with those structures which ordinarily develop into conidiophores. All cells involved in the development of the perithecium initial are uninucleate. The recurved terminal portion divides into three cells (fig. 3, H, J, Q), the middle or subterminal one of which becomes the oogonium. This very characteristic 3-cell arrangement is distinguishable in water mounts of fresh material as well as in stained sections.

#### EXPLANATORY LEGEND FOR FIGURE 2

- A.—Mature perithecium.  $\times 250$ .
- B.—Photomicrograph of section through an aggregation of perithecia in various stages of development.  $\times 100$ .
- C.—Photomicrograph of section through a young perithecium, showing oogonium in its initial binucleate stage.  $\times 530$ .
- D.—Same, with oogonium in 4-nucleate stage.  $\times 530$ .
- E.—Same, after the oogonium has divided into 2 binucleate cells.  $\times 530$ .

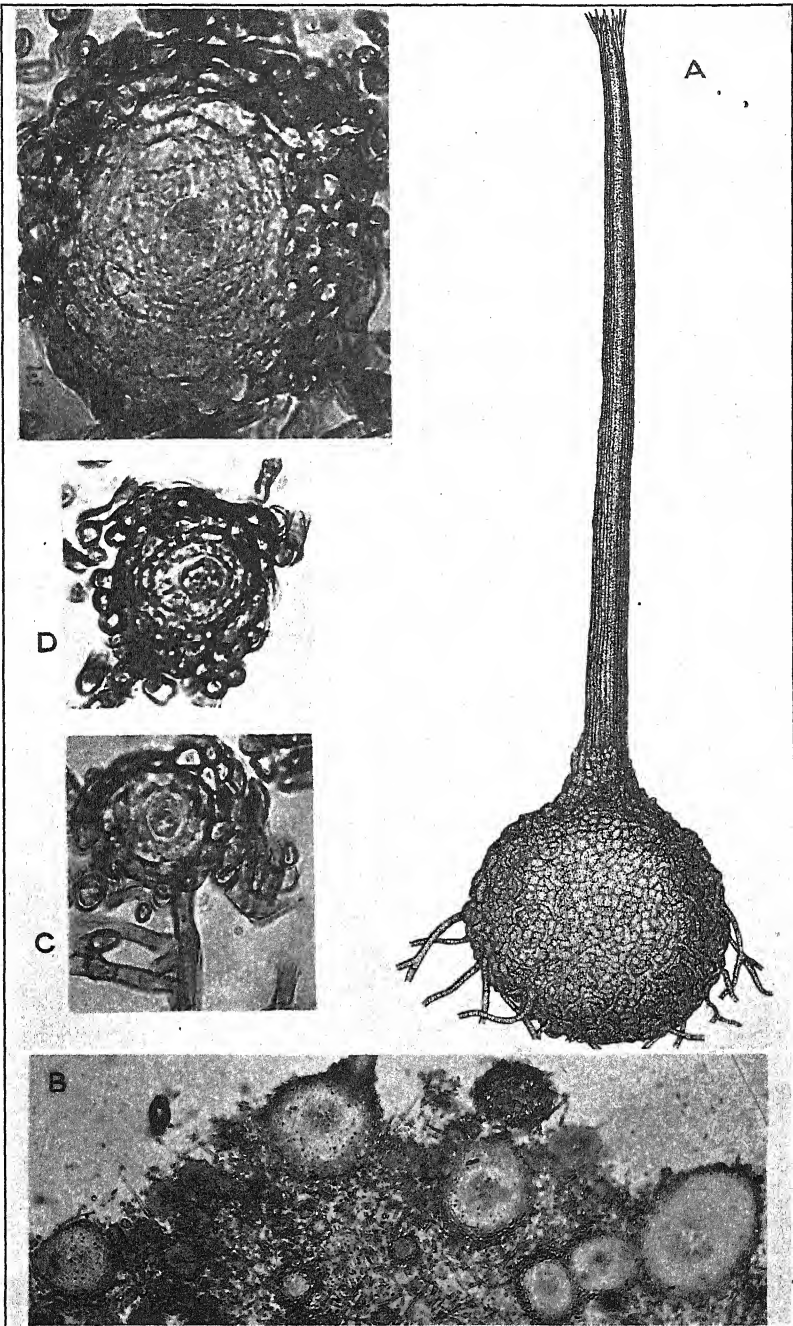


FIGURE 2.—Perithecia of *Ceratostomella fimbriata*  
For explanatory legend see opposite page

The antheridium is the first lateral branch formed below the oogonium. (Fig. 3, A to C, I to O.) Structurally it is identical with the branches formed later from the same and lower nodes of the perithecial stalk. (Fig. 3, R, S.) It is strange that the recurved oogonium should display such intimate contact with this particular organ, for apparently a fusion between the two structures rarely if ever occurs. Certainly there is no proof from the writers' preparations that a nucleus from the antheridium passes into the oogonial cell, either directly or through the terminal cell. On the other hand, there is evidence that a nucleus from the terminal cell of the ascogone may migrate into the oogonium, a phenomenon comparable to that believed by Faull (8) to occur in *Laboulbenia chaetophora* Thax.

It has been observed in two instances that two nuclei are present in the terminal cell at a very early stage. (Fig. 3, Q, and fig. 4, A.) This might suggest that the terminal cell acts as a trichogyne in conjugation with the antheridium, but evidence of such a conjugation is entirely lacking. The few clear cases showing an actual fusion appear to involve sterile structures and are unaccompanied by nuclear activity. (Fig. 3, V to X.) Figure 4, A, especially might be adduced as evidence that the oogonium is fertilized by a nucleus from the terminal cell. Here the wall separating the tip cell from the oogonium is incomplete, while the two identical nuclei in the tip cell are probably the result of a recent nuclear division. In later stages a single nucleus is found in the tip cell, which frequently persists without loss of turgor for some time after the paired nuclei are present in the oogonium. (Fig. 3, T, and fig. 4, B, F.)

The presence of a 3-celled structure previous to the origin of the paired nuclei, as shown in Figure 3, H, J, should be emphasized for the reason that it resembles very closely the supposed 3-celled ascogone of *Sphaerotheca castagnei* Lév., which, according to Hein (17, p. 400 and fig. 38), is formed after fertilization. These structures in *Ceratostomella fimbriata* can be seen in live material before there has been any possible fusion of oogonium with antheridium; and if the 3-celled ascogone is truly a sporophytic growth, it must be concluded that the origin of the paired nuclei does not depend upon the migration of a nucleus from an antheridium.

In Figure 3, Y, is represented a curious and conspicuous structure that is characteristically present at an early stage in perithecial development. Its appearance at once suggests a trichogyne. Perhaps it is a vestige of an ancestral copulatory organ, comparable to the trichogyne of some alga or lichen. On the other hand, were it not for the fact that the organ (fig. 3, Y, *a*) is so frequently present at just

#### EXPLANATORY LEGEND FOR FIGURE 3

A-H.—Initial organs of perithecial stalk drawn from live material; *a*, antheridial, and *b*, oogonial branch.  $\times 700$ .

I-O.—Initial organs drawn from stained sections. The terminal recurved portion in each case is the oogonial branch.  $\times 1,000$ .

P.—Two perithecial initials involved in a single coil.  $\times 1,000$ .

Q.—Perithecial initial showing 3-celled ascogone. Fertilization results from the migration of one nucleus from cell *b* into the oogonium, *c*; antheridium probably at *c*.  $\times 1,000$ .

R, S.—Later stage, showing first outgrowth of hyphae that envelop the oogonium.  $\times 1,000$ .

T.—Perithecial initial showing 3-celled ascogone with first layer of enveloping hyphae; oogonium shown at *a*, with tip cell (*b*) and basal cell of ascogone (*c*).  $\times 1,000$ .

U.—Young perithecium showing oogonium (*a*) after first nuclear division.  $\times 1,000$ .

V, W.—Aborted perithecia; cell fusions at *a* involve sterile structures.  $\times 1,000$ .

X.—Same; *a* and *b* show adjacent sections of same structure.  $\times 1,000$ .

Y.—Perithecial initials at *a*, *b*, and *c*, showing an accessory organ curiously suggesting a trichogyne.  $\times 1,000$ .

this stage, it might be regarded as simply a modified or aborted conidiophore, such as may frequently be seen growing from the superficial layers of older perithecia. It seems sufficiently clear that this appendage is not concerned with fertilization. The structure can not be identified in later stages and is apparently absorbed in the growing perithecium.

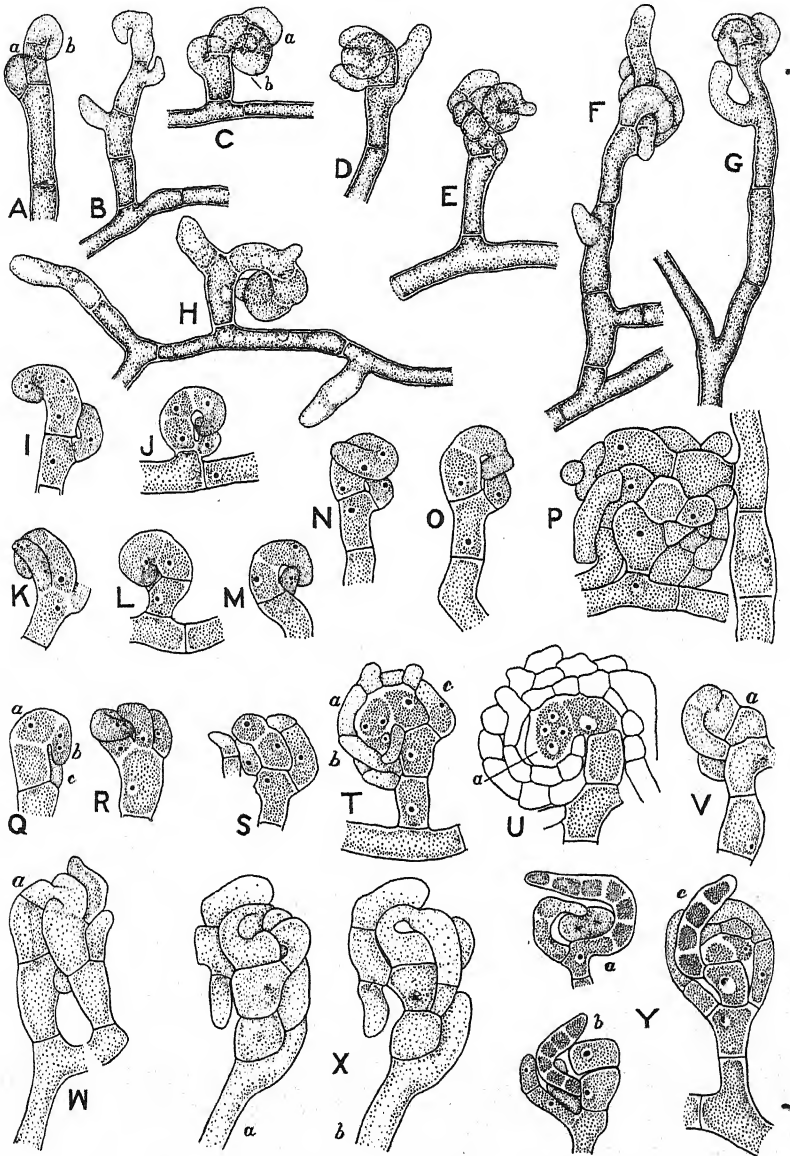


FIGURE 3.—Initiation of perithecia in *Ceratostomella fimbriata*  
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## MULTIPLICATION OF ASCI WITHIN THE PERITHECIUM

As the peritheciium enlarges externally by new hyphal growth the inner cavity enlarges, apparently by digestive action. (Fig. 4, I, and fig. 5.) · Dissection of a live peritheciium reveals activity in the cavity resembling yeast or bacterial fermentation in such a striking manner that the presence of a parasite was at first suspected. Later observations led to the discovery of a method of ascus multiplication that, judging from previous accounts of *Pyrenomyces* development, appears to be peculiar to *Ceratostomella fimbriata*.<sup>4</sup> In the following paragraphs an attempt will be made to trace the course of development of the oogonium and of the cells that result from its division.

The subterminal cell of the perithecial stalk becomes binucleate before any enveloping hyphae are in place, and this binucleate condition persists up to the time of ascus formation. No nuclear fusion occurs in the oogonium, as claimed by Harper and others (11, 15, 17) for other Ascomycetes. The only fusion of nuclei occurs in the young asci.

The oogonium forms a core for the young fruiting body (fig. 3, T), the nuclei and cell plasm become separated from the wall, and the protoplast lies free in the cavity of the peritheciium (fig. 4, B, C). The protoplast remains undivided for a brief time after the first layers of enveloping hyphae are in place. (Fig. 2, C, D; fig. 3, T; and fig. 4, B to D.) Nuclear divisions then occur, resulting in a cell with four to eight nuclei. (Fig. 3, U, and fig. 4, E to H, J, K.) The 4 to 8 nucleate body then undergoes a division or fragmentation (fig. 2, E; fig. 4, I, L; and fig. 6, A to C), and further division of the daughter cells and their progeny results in a multitude of apparently naked cells embedded in a nutrient medium and entirely detached within the enlarging perithecial cavity (fig. 5, and fig. 6, D).

The division of the daughter cells, following fragmentation of the oogonium, takes place in the binucleate condition (fig. 6, E, *a*), so that cells with four nuclei are frequently found before the constriction is complete (fig. 6, E, *b* to *d*). Later, certain of these cells fail to undergo any further division; the two nuclei fuse and the cell enlarges to form a young ascus. (Fig. 6, F, G, H.) The various stages in the nuclear fusion, as shown in Figure 6, J, are observed

<sup>4</sup> Some cytological preparations have been made of *Ceratostomella (Endoconidiophora) coerulea* (Münch) Clements and Shear, and these indicate that an essentially similar method of ascus multiplication occurs in this species.

## EXPLANATORY LEGEND FOR FIGURE 4

A.—Perithecial initial showing fertilization occurring simultaneously with the appearance of the first enveloping hyphae; constriction cutting off the tip cell from the oogonium at *a* (incomplete).

B.—Young peritheciium showing 3-celled ascogone after the tip cell (*b*) is separated from the oogonium (*a*).

C.—Same stage as B; either the tip cell is not shown or in this case it has become the oogonium.

D.—Early stage with the old hyphal wall still intact about the oogonium at *a*; terminal cell of ascogone not shown.

E.—Later stage, after nuclear divisions have occurred in the oogonium.

F.—Early stage with the three cells of the ascogone still visible. The significance of the apparently degenerate nuclei in the oogonium at *a* is not clear.

G.—Young peritheciium after first nuclear division of the oogonium.

H.—Same, showing oogonium just before cell division.

I.—Same, following the first fragmentation.

J.—Oogonium in 4-nucleate stage.

K.—Oogonium with eight nuclei previous to fragmentation.

L.—Ascogenous cells resulting from division of the oogonium. A nuclear division has occurred in the daughter cell *a*, and cell *b* shows nuclei in process of division. Dotted lines in J, K, and L indicate the limits of the perithecial cavity.

All figures  $\times 1,000$ .

regularly and clearly. The remaining cell of the recently divided pair will undergo a further division (fig. 6, F, c), and one of its progeny will form an ascus. It is natural to assume that each daughter cell normally receives nuclei of diverse lineage. The only evidence of the relationship of paired nuclei must be based upon their observed conjugate division and their relative positions at the time of fixation, and upon this basis it can not be said with certainty that the two nuclei which fuse in the ascus are not sister nuclei.

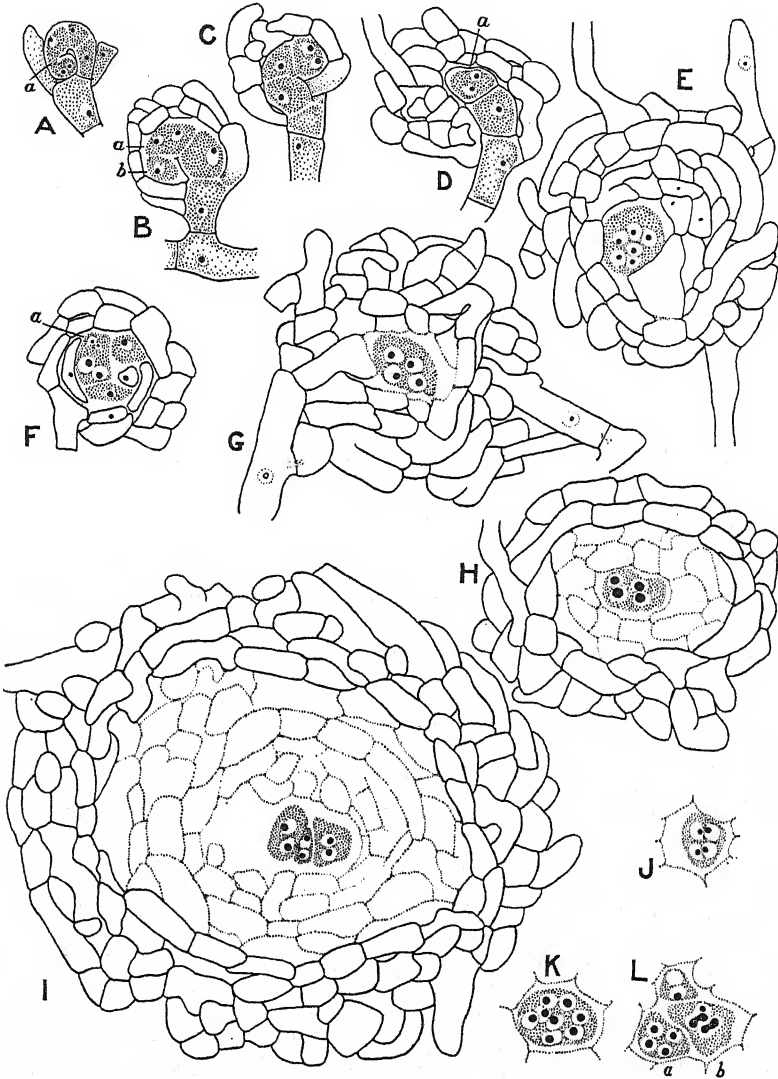


FIGURE 4.—Development of perithecia in *Ceratostomella fimbriata*  
For explanatory legend see opposite page

Free cells of various sizes with 1, 2, and 4 nuclei can be found intermingled in the perithecial cavity even before the fruiting body is half mature. (Fig. 5.) These young ascogenous cells may cling together in series of two to four, but they are without hyphal connection with the wall of the perithecium. In those cases in which

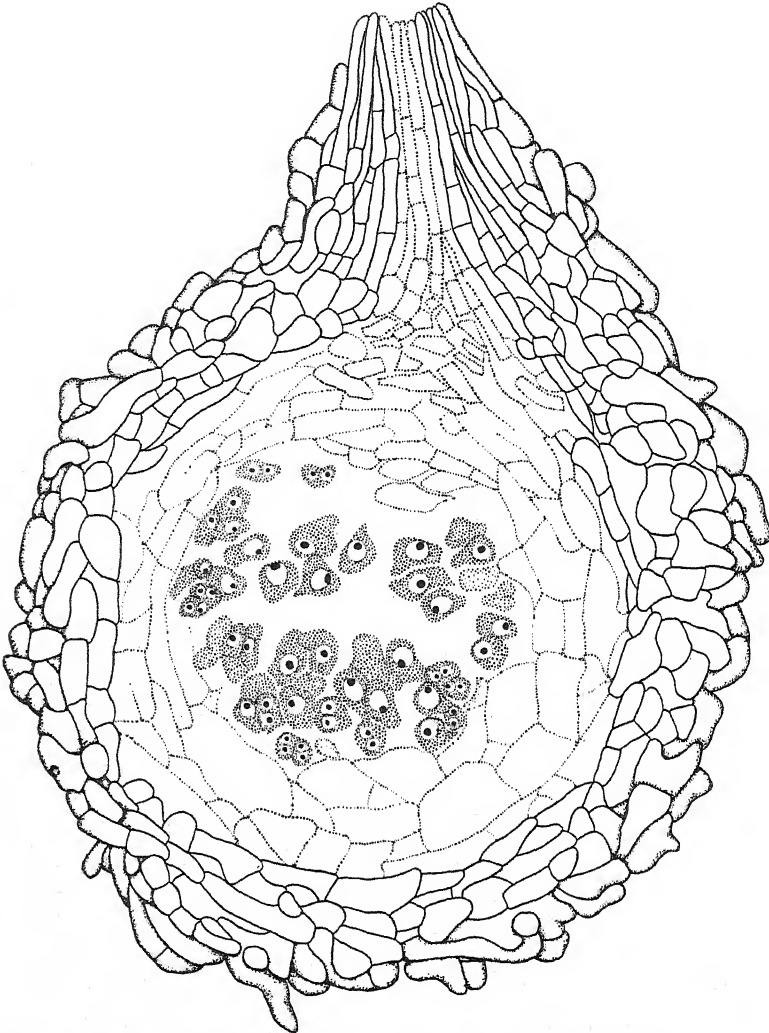
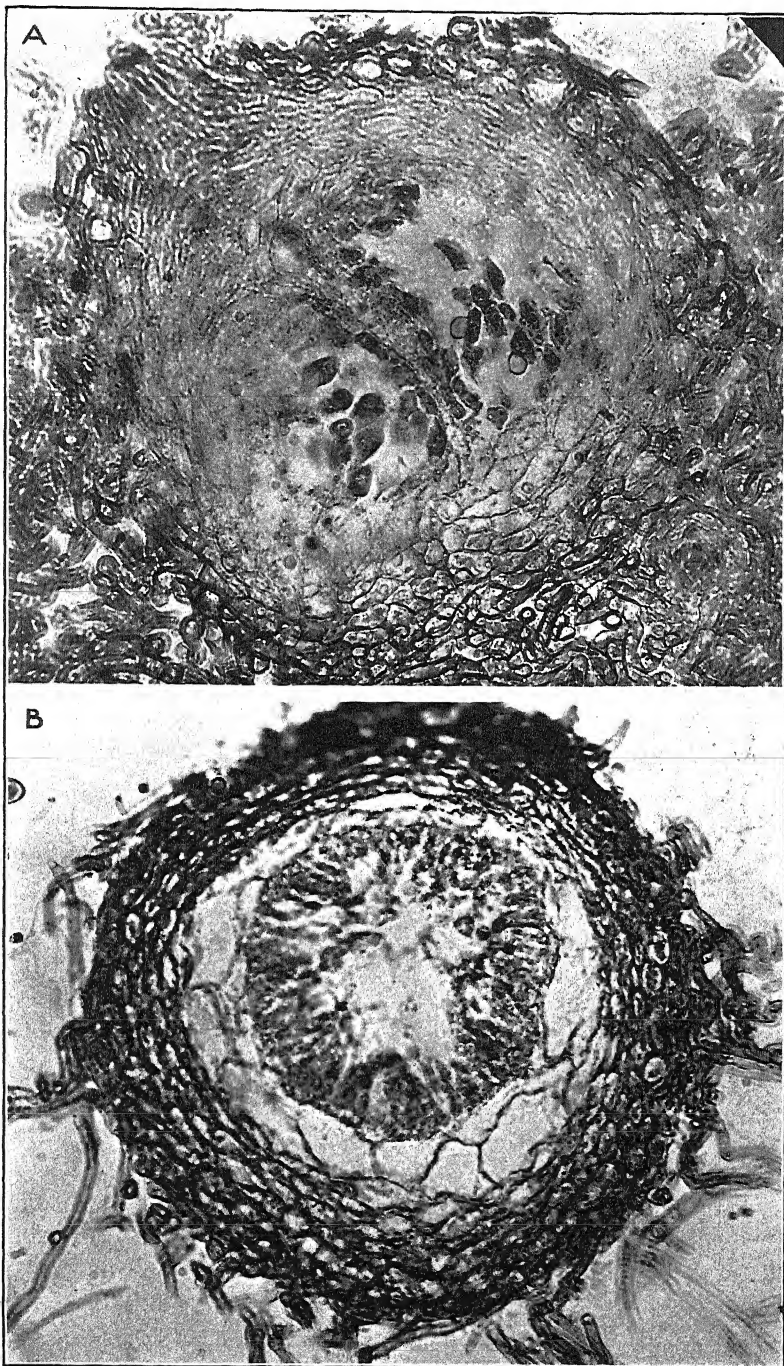


FIGURE 5.—Perithecium of *Ceratostomella fimbriata* at beginning of beak formation, showing zonation of perithecial walls and arrangement of ascogenous cells in the cavity.  $\times 700$

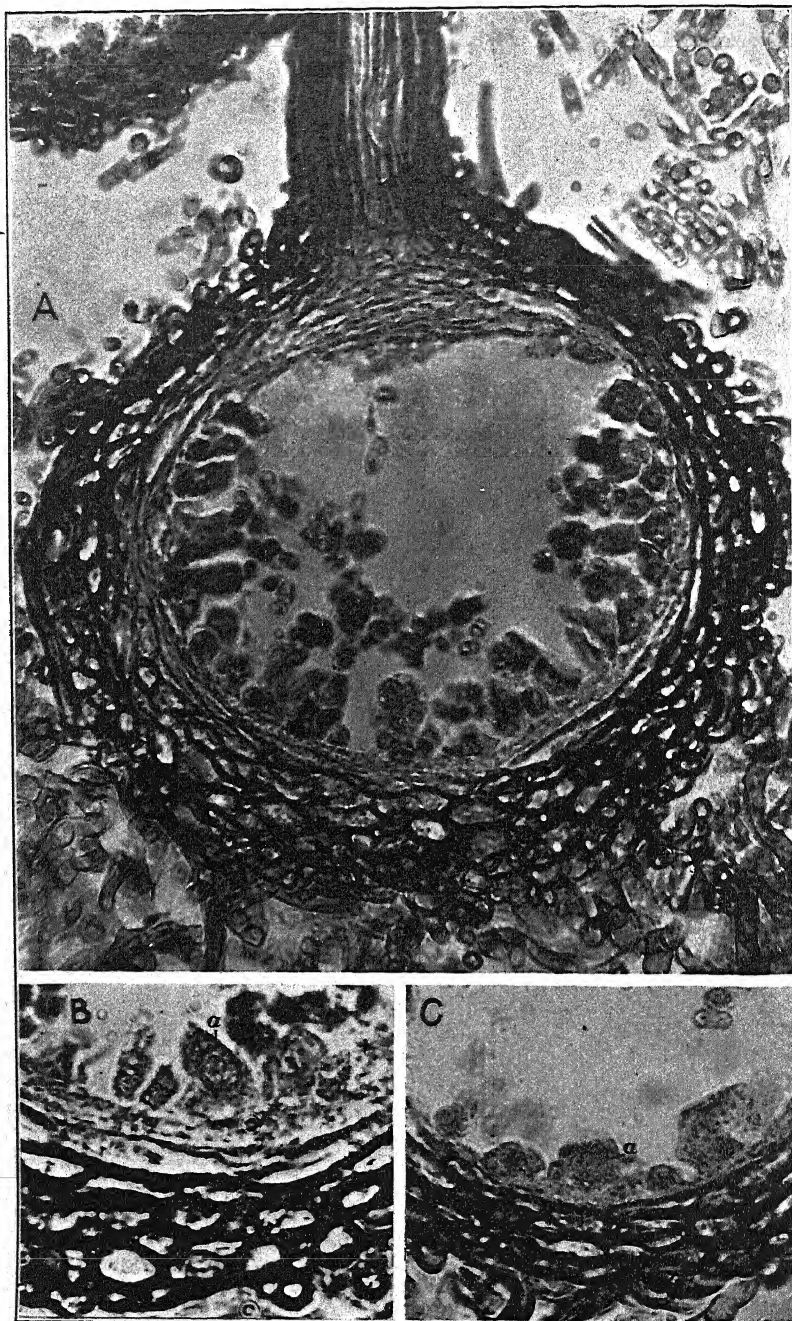
cells cling together they do so by a very slender filament. (Fig. 6, E, d.) The connected cells frequently form short chains not wholly unlike the series of asci described by Faull (8, fig. 38, pl. 38) for some of the Laboulbeniales.

Contrary to claims made by Elliott (?), no structures resembling ascogenous hyphae are found at any stage in the development of





Perithecia of *Ceratostomella*: A, Photomicrograph of section showing coalescence of immature perithecia of *C. fimbriata*; the original wall of separation is nearly consumed by digestive processes in the two cavities.  $\times 400$ . B, Photomicrograph through section of immature perithecium of *C. adiposum* showing radially disposed columns of ascogenous cells.  $\times 400$ .



Arrangement of asci in perithecia of *Ceratostomella fimbriata*: A, Mature fruiting body with most of contents collapsed against the walls of the perithecium.  $\times 500$ . B, C, Detail showing asci with internal vesicle at  $\alpha$ .  $\times 660$ .

the perithecium. Instead of forming ascogenous hyphae, the protoplast of the oogonium breaks up into binucleate ascogenous cells which after a series of further divisions develop into asci. (Fig. 6.) The absence of cell walls and of cross walls permits the dividing protoplasts to separate easily. (Fig. 6, G.) Thus for the most part the numerous cells are wholly detached from one another but frequently remain in apposition, forming irregular series of two to four cells. (Fig. 6, F, G, H.)

The first stage of division of the ascogenous cells is characterized by the appearance of a simple cleft, as shown in Figure 6, E. The fissure increases until the protoplasmic connection is broken. (Fig. 6, G.) The cells in process of division form structures resembling ascus hooks, or what are often referred to as "croziers" (26). (Fig. 6, E.) It is clear that Varitchak (26) may easily have been misled into supposing that true ascus hooks are formed in *Ceratostomella piceae* Münch. With few exceptions his figures show structures that appear identical with those found in *C. fimbriata*, in which there can be no doubt as to the mechanism of the dividing cells. There are no ascogenous hyphae with croziers, but simply free cells dividing by a peculiar process of constriction. (Fig. 6, E.) The type of septation shown in Varitchak's Figure 2, *g*, Plate 10, has not been observed in *C. fimbriata*, although it is not inconceivable that a division might occasionally take place in such a fashion. His figures do not indicate that this is the usual procedure in *C. piceae*. In *C. fimbriata*, a daughter cell is rarely abstricted with a single nucleus, leaving three nuclei in the parent cell. (Fig. 6, I, *b*.) A cell is sometimes found dividing as shown in Figure 6, I, *c*, where an extra nuclear division has taken place in advance of the separation of the daughter cells. Such irregularities in cell mechanics might well be anticipated.

After the initial stage the perithecial wall assumes two well-marked zones—an inner zone of very thin-walled colorless cells that are being rapidly digested, and an outer zone of relatively thick-walled pigmented cells where new layers are being constantly added. (Fig. 5.) Neither paraphyses nor periphyses are found at any stage in the development of the perithecium.

The smallest and most recently formed ascogenous cells are found normally in close proximity to the lining of the cavity. (Pl. 2, A.) However, they are disposed at all angles and are often found in the center, remote from the perithecial wall. (Fig. 5.) It is important to note that the digestion of the inner wall of the perithecium appears to take place equally in all directions, so that there is no possibility of the central core of ascogenous cells retaining their original connection with the crown of the perithecial stalk. There is thus entire absence of hyphal connection with the body of the perithecium, except, perhaps, as may rarely result through secondary attachments of the isolated protoplasts. The semiliquid débris resulting from the recently digested inner cells of the perithecium forms an excellent medium for orientation of the unvalled cells. The absence of a cell wall possibly facilitates the nutritive relation of the ascogenous cells with the medium in which they are embedded. This apparently naked condition begins with the single-celled oogonium and is maintained until the asci are in process of maturation, at which time a new wall is formed endogenously, as will be shown later.

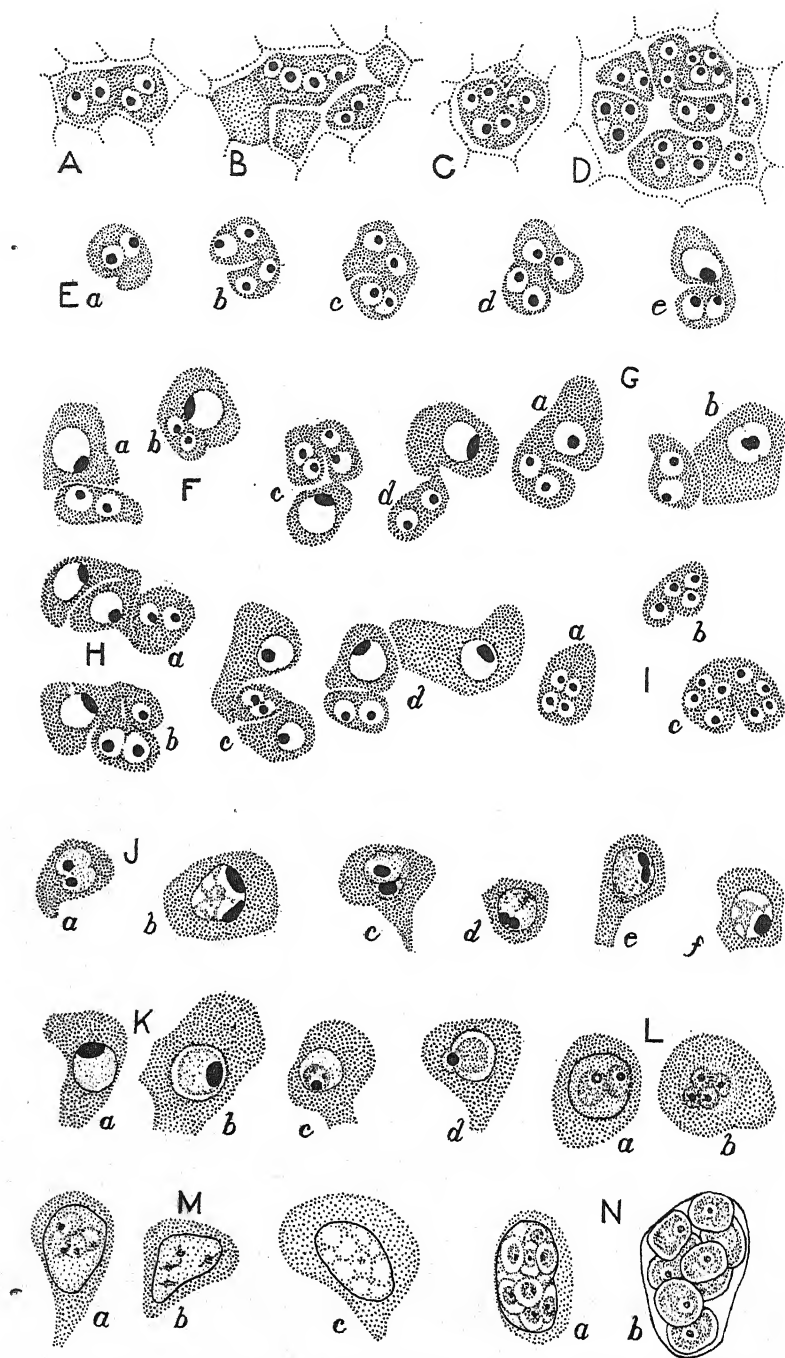


FIGURE 6.—Division of ascogenous cells and maturation of asci in *Ceratostomella fimbriata*  
For explanatory legend see opposite page

The structure of the young asci and of the dividing cells, as shown in Figure 6, A to M, can not readily be seen by dissecting the live perithecia in water. The tiny, unwallled, and almost colorless cells become dissolved or lose their form completely in water. But they can be demonstrated clearly by crushing or dissecting the perithecia in Amann's solution with nigrosine as a stain.<sup>5</sup> This solution both kills and fixes the material, and the naked protoplasts stain a faint blue with the nigrosine.

The chains of ascogenous cells in *Ceratostomella adiposum* (Butler) Sartoris appear as simple columns of two or more cells regularly disposed about the interior of the perithecium as described by Sartoris (24). An examination of some of Sartoris's slides and of fresh material of *C. adiposum* confirms his observation that asci are formed by a simple terminal abstriction of binucleate cells. Sartoris places no emphasis on the fact that these columns of ascogenous cells are not growing out of the wall of the perithecium or from any basal hyphae lining the cavity, but are, like the ascogenous cells of *C. fimbriata*, disposed unattached within the perithecial cavity and are in fact growing toward the periphery of the perithecium. (Pl. 1, B.) The ascogenous cells appear to consume the body of the perithecium by continual growth from the center outward. (Fig. 5; pl. 1, A; and pl. 2, A.) In other words, the inner wall of the perithecium is rapidly digested, perhaps by an enzymatic action on the part of the rapidly multiplying free cells, which continuously encroach upon the limits of the cavity.

#### MATURATION OF ASCI

The first asci begin to mature at about the time the perithecium has attained approximately one-half its full diameter and when the beak or neck of the perithecium has just begun to form. (Fig. 5.) From this time on, ascogenous cells in all stages of development can be seen in the same perithecium, the younger cells prostrate and lying at all angles near the wall of the cavity.

The preponderance of asci in the fusion nucleus stage (fig. 6, J, f) indicates that this stage persists for a relatively long period, while the subsequent 2, 4, and 8 nucleate stages occur in rapid succession. The nuclear behavior from the beginning of the first division to the complete delimitation of ascospores is very obscure; only rarely can

<sup>5</sup> The following formula was used in this work: 1 part concentrated lactic acid, 1 part phenol, 1 part 95 per cent alcohol, 2 parts glycerin. Add a few crystals of nigrosine.

#### \* EXPLANATORY LEGEND FOR FIGURE 6

A-D.—Early stages in the fragmentation of the oogonium. Cells with four nuclei are prepared for another cell division. Dotted lines indicate limits of perithecial cavity. (Refer to fig. 4, I.)

E.—Stages in division of ascogenous cells; *a*, before nuclear division; *b-e*, after nuclear division, showing separation of daughter cells; two nuclei in upper cell of *e* have fused in forming a young ascus.

F, G.—Further proliferation of ascogenous cells; in each case one daughter cell forms an ascus while the second cell prepares for another division.

H.—Linkage of asci and ascogenous cells after successive divisions.

I.—Irregularities in cell division; *a* is probably the result of a division such as is shown in *c*; *b*, unequal division.

J.—Nuclear fusion in the ascus (*a-e*); young ascus (*f*).

K.—Nuclear contraction in the ascus; *a*, before contraction; *b-d*, stages in contraction showing origin of the ascus vesicle. It appears in *d* that the nucleolus is being ejected from the vesicle.

L.—First nuclear division in the ascus; wall of the vesicle distinct in *a* and very faint in *b*.

M.—Further expansion of the nuclear membrane to form the wall of the ascus; 4-nucleate stage in *a*, faint spindle-shaped structures in *b*; *c* indicates the obscure nuclear behavior during spore delimitation.

N.—Maturation of ascospores; *a* shows ascus and eight young spores with outer integument of cytoplasm still adherent to the ascus; *b*, ascus with eight mature spores.

All figures  $\times 1,500$ .

the several stages shown in Figure 6, L, M, be seen clearly, the outline of the vesicle alone remaining distinct. Most frequently the asci have the appearance indicated in Figure 6, M, *c*, from which it would appear that the nuclei during this period do not usually enter a resting stage until the eight spores are formed.

The first nuclear division in the ascus is characterized by the development of an ascus vesicle, the wall of which appears to be the membrane of the fusion nucleus. (Fig. 6, L, *a*.) All three divisions and the later delimitation of ascospores, therefore, take place within the nuclear wall. (Fig. 6, K to N, and pl. 2, B, C.) In some cases the first indication of the ascus vesicle is the contraction of nuclear substance, resembling a nuclear plasmolysis. (Fig. 6, K, *b*, *c*.) In Figure 6, K, *d*, there is evidence that the nucleolus is ejected from the vesicle just preceding the first nuclear division, but there is no reason to conclude that this is the usual procedure. The regular disappearance of the nucleolus may be accounted for in some other fashion. That the nucleolus at this stage corresponds to a plasmosome (27) is indicated by its affinity for plasma stains rather than chromatin stains, and the possibility should not be overlooked that it may perform some active function in the formation of the vesicle wall and of the cytoplasm within the vesicle.

The vesicle expands rapidly and continuously during the period of nuclear divisions (fig. 6, L, M) and has reached nearly the dimensions of an ascus by the time the eight young spores are formed (fig. 6, N, *a*). The ascospores undergo a further increase in size, and this expansion usually succeeds in rupturing the membrane, leaving the spores free within the perithecium, from which they are ejected through the beak in a mucilaginous coil. The extreme fragility of the mature ascus wall is indicated by the fact that asci can rarely be found with a full complement of eight mature spores. (Fig. 6, N, *b*.) Those asci observed by dissection of perithecia in water have partially broken walls or contain immature spores.

So far as the writers are aware, no one has reported previously a case in which the wall of the fusion nucleus becomes the wall of the ascus. The procedure is doubtless peculiar to those species of *Pyrenomyces* whose asci in their younger stages are without a definite wall. The nuclear contraction just preceding the first division is perhaps comparable to the contraction phase frequently described in the literature on ascus cytology, but in such cases the nuclear membrane does not persist as in *Ceratostomella fimbriata*. In regard to the nuclear divisions in the ascus, Elliott (7, p. 421) observed that "these divisions occur within the nuclear-plasm, which in many cases is distinctly separate from the cytoplasm of the ascus." He does not account for the origin of the ascus wall, doubtless for the reason that he failed to observe the essentially naked condition of the young asci.

In describing the life history of the red alga *Polysiphonia violacea* Grev., Yamanouchi (28, v. 41, p. 431) writes as follows:

It is a remarkable fact that the membrane of the original nucleus in the tetraspore mother-cell persists through the two mitoses which have just been described. The area included by this membrane increases in size with the growth of the cell.

In a later paper Yamanouchi (28, v. 42, pl. 27) illustrates this stage in the development of the tetraspore mother cell of *Polysiphonia*.



The phenomenon apparently has little relation to the origin of an ascus wall in *Ceratostomella*.

Sartoris (24) apparently overlooked the occurrence of ascus vesicles in *Ceratostomella adiposum*, although they appear clearly on his slides. In a personal discussion of this point Sartoris expressed himself as favorably disposed to the interpretation that an ascus wall is formed endogenously in *C. adiposum*. It is true that a vesicle appears to be absent in many of the developing asci in both *C. fimbriata* and *C. adiposum*, but it also appears that many groups of ascospores mature in complete absence of a retaining wall. It was this condition which probably misled mycologists for a long time in supposing that the perithecia of these fungi were pycnidia, producing numerous free pycnidiospores.

### DISCUSSION

An attempt has been made to throw additional light on a much neglected field of fungus morphology—early perithecial development in the Sphaeriales. The results of a previous study on the development of *Ceratostomella fimbriata* by Elliott (7) indicate that the organism conforms to the familiar views already held regarding Ascomycete morphology. The present investigations, however, do not support Elliott's conclusions in many particulars.

In the material studied by the writers it is clear that both antheridium and oogonium are borne on the same hypha. (Fig. 3, A to O.) If a conjugation takes place at all it is between structures genetically separated by not more than three cell divisions. It is believed, however, that fertilization in *Ceratostomella fimbriata* is accomplished by the migration of a nucleus from the terminal cell of the ascogone into the subterminal cell or oogonium. (Fig. 3, I, Q, and fig. 4, A.) In those species of Ascomycetes where a union of male and female elements can be demonstrated by the methods of hybridization, the question of fertilization to that extent may be regarded as closed (4, 22); but in those species, such as *C. fimbriata*, where a like method appears unavailable, no positive conclusions can be reached. Even though a conjugation of two elements can be adequately demonstrated, proof is yet lacking that this constitutes an effective sexual act.

Dangeard (3) speaks of the antheridium of some Pyrenomycetes as a "trophogone," inferring thereby that a fusion of a "trophogone" with an oogonium has only a nutritive value. However, convenience may justify the use of the term "antheridium," although it is supposed, in the absence of proof of nuclear migration from antheridium to oogonium, that a fusion of these structures is not sexually effective. In the case of *Ceratostomella fimbriata* there is no proof that antheridium and oogonium ever fuse, although the two structures can be easily identified at the stage where such a fusion would be expected to occur. Yet there seems to be no reason to doubt the numerous reports of Harper and his students wherein a true fertilization is described for a number of species of Ascomycetes. Attention should be called only to the situation in many Phycomycetes where development of the oogonium proceeds whether or not copulation takes place (5, 9).

The absence of a nuclear fusion in the oogonium of *Ceratostomella fimbriata* may indicate further that the paired nuclei have not resulted

from copulation but have developed spontaneously in the terminal region of the ascogone. (Fig. 4, A.) Gwynne-Vaughan and Williamson (11) have recently reaffirmed a conviction that nuclear fusions occur following conjugation in *Pyronema*. It is a familiar fact, however, that nuclear fusions at this stage have been seriously questioned by various observers, even in *Pyronema*.

The oogonium of *Ceratostomella fimbriata* may be compared to that of species of *Endomyces*, or to that of *Sphaerotheca castagnei*. In *Endomyces magnusii* Ludwig (10) the oogonium develops directly into a single ascus. In *S. castagnei* (14, 17) the oogonium by division forms a series of three to five cells, only one of which normally becomes an ascus. In *C. fimbriata* the oogonium, by its division and by further division of its progeny, forms a multitude of cells all destined to become asci.

Three unique features in the process of ascus multiplication in *Ceratostomella fimbriata* deserve particular emphasis. One consists in the complete absence of any hyphal connection between the body of the perithecium and the ascogenous cells that occupy its interior. (Fig. 5.) Such a wholly unorthodox situation appears less remarkable when it is recalled that the ascogenous cells are not derived from ascogenous hyphae but result from a progressive cell fission that begins with the original protoplast in the subterminal cell of the ascogone. (Fig. 6, A to D.) We need further to recall that the need for connecting hyphae in obtaining nutrients is obviated by the condition that the ascogenous cells are themselves embedded in a nutrient medium.

Varitchak (26) speaks at considerable length of the ascogenous hyphae in *Ceratostomella piceae*, whereas his figures show practically no evidence of these structures, and in view of the observations made with respect to *C. fimbriata* it appears doubtful if ascogenous hyphae are formed in *C. piceae*. Varitchak regards the crescent-shaped and recurved cells in the perithecium of *C. piceae* as ascus croziers, supposing that the penultimate portion becomes an ascus while the ultimate and antepenultimate cells fuse to form a second one, such being the manner in which a crozier is supposed to function. Nevertheless, his figures indicate that asci may be formed by a simple cell fission essentially identical with the process in *C. fimbriata*.

The division of ascogenous cells in *Ceratostomella adiposum* differs from that in *C. fimbriata* only in appearance. In both species there is a simple fission of binucleate cells. In *C. fimbriata* the progress of fission produces curious structures superficially resembling ascus croziers, and the manner in which the young asci cling together (fig. 6, H) suggests the serial arrangement of asci in *Laboulbenia chaetophora* (8). In *C. adiposum* such hooked structures are seldom found; the dividing cells remain in apposition along the entire plane of fission, so that neat rows of cells are formed that stand side by side and give a false appearance of a hymenium lining the whole inner face of the perithecium. (Pl. 1, B.)

A second feature of particular interest is the apparently naked condition of the ascogenous cells and young asci. The absence of a cell wall begins with the primary ascus or oogonium (fig. 4, G) and appears first as a plasmolysis or shrinkage of the protoplast away from its wall. The delicate membrane that was once the hyphal wall can



still be seen as indicated in Figure 4, D, *a*, but from this point on the tiny protoplasts multiply in an essentially naked condition until a new wall is formed endogenously in the ascus. (Fig. 6, K to N.) There is no indication that the protoplasts may be bounded by a plasma membrane of a more definite nature than a simple surface film. The frequently angular shape of the dividing cells indicates a considerable degree of firmness or rigidity of the protoplasm independent of any surface film. The intimate contact of the unwalled cells with the semiliquid debris resulting from the digestion of the inner cells of the perithecium must eliminate any need for hyphal connections with the base of the fruiting body.

Another feature in the life history of *Ceratostomella fimbriata* which is unknown among other ascomycetous fungi so far studied is the endogenous formation of an ascus membrane. By this process the wall of the fusion nucleus becomes the wall of the ascus. Among the Ascomycetes that have been studied cytologically, it has been frequently reported that the spindles are formed within the nuclear wall during the meiotic divisions. In no case, however, has it been shown that the original membrane of the fusion nucleus persists through the three divisions and finally incloses the eight ascospores, although this appears to be the usual procedure in *C. fimbriata*. (Fig. 6, K to N.) If one considers that all the young asci here have developed in the absence of a wall, it may appear that such a persistence of the nuclear membrane has a peculiar utility for this and related species.

There are certain theoretical objections to the view that the ascospores are delimited within the membrane of the fusion nucleus. The interpretation does not accord easily with the view that cytoplasm and nucleoplasm are multiplied independently of each other. It does accord with the view that cytoplasm may be a product of nuclear activity. It is of course possible that the wall of the vesicle (fig. 6, L, and pl. 2, B, C) did not originate as the nuclear wall itself; there are cytologists who would perhaps deny that a nucleus has a wall of a more definite nature than a simple film separating one cell phase from another. It is possible that a new wall is produced in the cytoplasm closely surrounding the nucleus. The stages shown in Figure 6, K to M, however, indicate fairly conclusively that the nuclear membrane becomes the wall of the ascus.<sup>6</sup>

Seeking among other fungi for a parallel to the process of free cell division in *Ceratostomella*, one finds what at first promises to be a close approach in *Monascus purpureus* Went, as described by Ikeno (18) in 1903. According to Ikeno, there occurs what is essentially a fragmentation of the ascogonium, or the aggregation of cytoplasm about certain nuclei, so that numerous isolated cells or "Cytoplasmaballen" are formed. These isolated cells undergo a further fission and ultimately form "sporangia" or asci of six to eight spores. Ikeno, supposing that the fungus was not a true Ascomycete, regarded the free cells as "spore mother cells."

A further search of the literature on *Monascus* shows a remarkable series of contradictions and reinterpretations (1, 19, 25), culminating

<sup>6</sup> In respect to the multiplication of asci by the division or fragmentation of naked and unattached cells the observations reported here are supported by those of Mittmann (21). Apparently due to a misinterpretation of the nucleus, Mittmann does not reach the same conclusion in regard to the origin of the ascus wall. The structure which she identifies as a nucleus is believed to be only a nucleolus, as will be shown clearly by comparison of her drawings with Figure 6 of this paper.

in the most recent new "interpretation" by Young (29) on *Monascus ruber* Tiegh. This paper gives an entirely new aspect to the Cytoplasmaballen of Ikeno, the author supposing that they are in fact ascospores in process of formation. Young does not account satisfactorily for the final multiplicity of asci, and her interpretation is not entirely convincing to one familiar with the sequences in the development of these organs. There is still a strong possibility that the earlier deductions of Ikeno may prove to be essentially correct.

One fact stands out conspicuously in most of the work done on the morphology of fungi of this type. This is the obscurity surrounding the early stages in the multiplication of asci. For most of the Sphaeriales these stages have been passed over lightly, it being supposed that ascogenous hyphae are formed comparable, for example, to those in *Pyronema* (11). It is becoming increasingly evident that the study of ascomycetous fungi should include a more careful examination of early stages of development and that classification should not follow too closely the characters of the mature fruiting body. It is possible that in the early proliferations of the ascogonium will be found characters of much value in indicating relationships among Pyrenomycetes.

Certain features in the life history of *Ceratostomella fimbriata* discussed in this paper may be regarded as unique among true Ascomycetes. They perhaps indicate some degree of affinity with the Hemiascomycetes. That these same features are essentially true also for *C. adiposum* has been borne out by limited observations on the latter species. It appears probable also that corresponding stages in the development of *C. piceae* as reported by Varitchak (26) (judging from his figures rather than his statements) differ only in detail from those in *C. fimbriata* and *C. adiposum*. The process of free cell fission in the perithecium as described here might well be regarded as an adequate basis for a new order of Pyrenomycetes. The process possibly is associated with the deliquescence of asci in the perithecium, a feature that distinguishes a considerable number of the Sphaeriales, but such an association can not be assumed to exist. Therefore, if a truly natural classification of fungi is an ideal worth attaining it will require a closer scrutiny of developmental processes than has been devoted to them in the past.

#### SUMMARY

Asexual reproduction in *Ceratostomella fimbriata* is accomplished by two conidial forms. Both types of conidia are abstricted within the sheath of the sporophore and are known as "endoconidia."

The fungus is one of a group of Pyrenomycetes notable for the early disintegration of ascus walls.

The organism is entirely homothallic. Antheridium and oogonium are borne on the same hyphal branch. A large number of single ascospore isolations have failed to reveal any evidence of sexual strains.

The antheridium is believed to be functionless, fertilization being effected by a nucleus arising in the terminal region of the ascogone.

The subterminal cell of the perithecial stalk normally becomes binucleate before any enveloping hyphae are in place, and this binucleate condition persists up to the time of ascus formation.

There is only one nuclear fusion in the life cycle; this occurs in the young asci.

The oogonium becomes the primary ascus cell; its wall dissolves, and it lies free in the cavity of the young perithecium.

As the perithecium increases in size by new hyphal growth a digestive action enlarges the inner cavity by a dissolution of the internal cells.

Neither paraphyses nor periphyses are formed.

No ascogenous hyphae are formed. Fragmentation of the oogonium and of its daughter cells results in a multitude of naked cells embedded in a nutrient medium and entirely detached within the perithecial cavity.

These ascogenous cells may cling together in series of two to four, but they are without hyphal connection with the perithecial wall or its base.

The method of division or constriction of the ascogenous cells gives the false appearance of an ascus hook or crozier.

The two nuclei in certain of these cells fuse at an early stage and this large definitive nucleus persists for a relatively long period, after which three divisions in rapid succession result in a cell of eight nuclei and the later delimitation of eight ascospores.

The first of the three nuclear divisions is characterized by the appearance of an ascus vesicle, the wall of which appears to be the membrane of the fusion nucleus.

Subsequent nuclear divisions and the delimitation of ascospores appear to take place within this membrane. The wall of the fusion nucleus therefore becomes the wall of the ascus.

Ascus walls are dissolved early, often before the spores are quite mature, so that a mature perithecium contains numerous free ascospores that are ejected through the beak in a mucilaginous coil when the perithecium is moistened.

The process of free cell division that results in a multiplication of asci may be a more accurate measure of the phylogenetic relationship of the genus than the characters now being employed.

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# VEIN SPOT OF THE PECAN CAUSED BY LEPTOTHYRIUM NERVISEDUM, N. SP.<sup>1</sup>

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## INTRODUCTION

Vein spot, apparently an unreported disease of the pecan (*Hicoria pecan* (Marsh.) Britton), appears to be one of the principal foliage diseases of pecans in the southwestern part of the United States. Demaree<sup>3</sup> collected the vein-spot fungus near Austin, Tex., in 1920, and at Ferriday, La., in 1929, and the writer has collected it on both native and cultivated pecans in Louisiana, Mississippi, Arkansas, and Texas. The disease has not been reported from the southeastern pecan belt, which includes Alabama, Georgia, Florida, South Carolina, and North Carolina.

## THE DISEASE

In the vicinity of Shreveport, La., vein spot first appears in May or June on the foliage of vigorous pecan trees, and in some orchards it causes the leaves of certain varieties to shed prematurely. The disease may attack the rachis, the petiole, or the veins, but the entrance of the fungus is always confined to the vascular system of the leaf. Infection may take place at or near the end of the midrib of a leaflet and extend down the midrib to its base (fig. 1), producing a narrow black necrotic streak. The discoloration of the leaf tissues seldom extends more than 2 mm or 3 mm on either side of the midrib. The infection may also extend from the main vascular bundle that runs lengthwise to the smaller lateral ones. When numerous small lateral bundles are killed the leaf tissue between them dies, forming large dead areas that may later involve the entire leaflet, causing it to drop. The fungus may attack either the terminal or the basal portion of the rachis and gradually extend to the opposite end (fig. 2), causing the leaflets to fall one at a time until finally the rachis, devoid of leaflets, is left hanging on the tree. In other instances, when either primary or secondary veins become infected, the fungus does not spread extensively, but causes a small oval-to-circular black spot. A leaflet may contain 8 or 10 of these black spots, which are sometimes not over 2 mm in diameter. The vein divides such spots into two almost equal parts. In some instances the fungus attacks the midrib of a leaflet and finally causes it to shed without interfering with the rest of the leaf (fig. 4), whereas in other instances it seems to attack the rachis, midrib, and vein simultaneously (figs. 1-3). In serious cases,

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<sup>2</sup> The writer wishes to acknowledge the helpful services of the following members of the Bureau of Plant Industry: C. L. Shear, for suggestions in the determination of the organism causing vein spot of pecans; John W. Roberts, for his criticism of the manuscript; Edith K. Cash, for assisting in translating the technical description into Latin; and J. B. Demaree, for many courtesies during the progress of the investigation.

<sup>3</sup> This information was obtained by the writer through correspondence with J. B. Demaree, pathologist, Division of Horticultural Plants and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

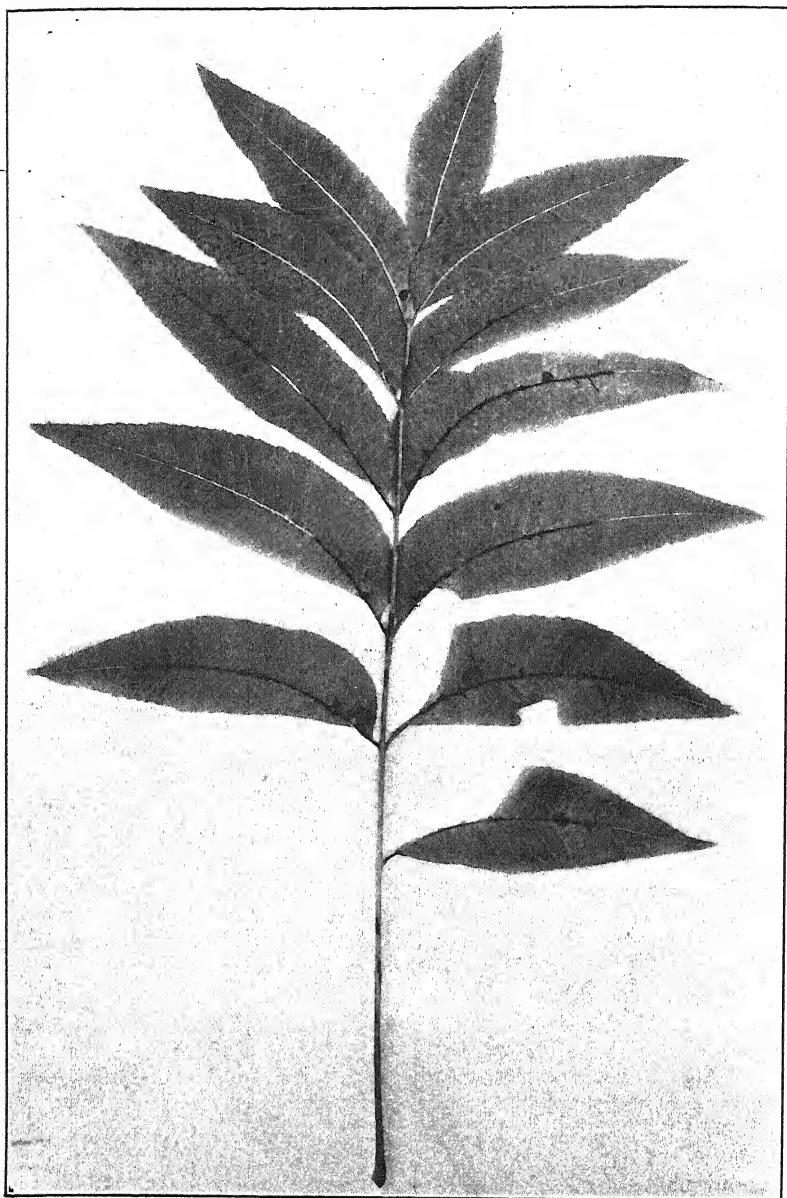


FIGURE 1.—Characteristic appearance of a pecan leaf attacked by the vein-spot fungus. The discoloration is confined strictly to the midribs and veins of the leaflets and to the petiole

such as occurred in the vicinity of Austin, Tex., the entire vascular system of many leaves is attacked; and severe defoliation results.

The spots which appear on the lower or ventral side of the leaves and four or five days later on the dorsal surface are yellowish at first and later brown, finally turning dark brown or black with yellowish margins. In the later stages of the disease the spots on both surfaces of the leaves have about the same appearance, except when fruiting

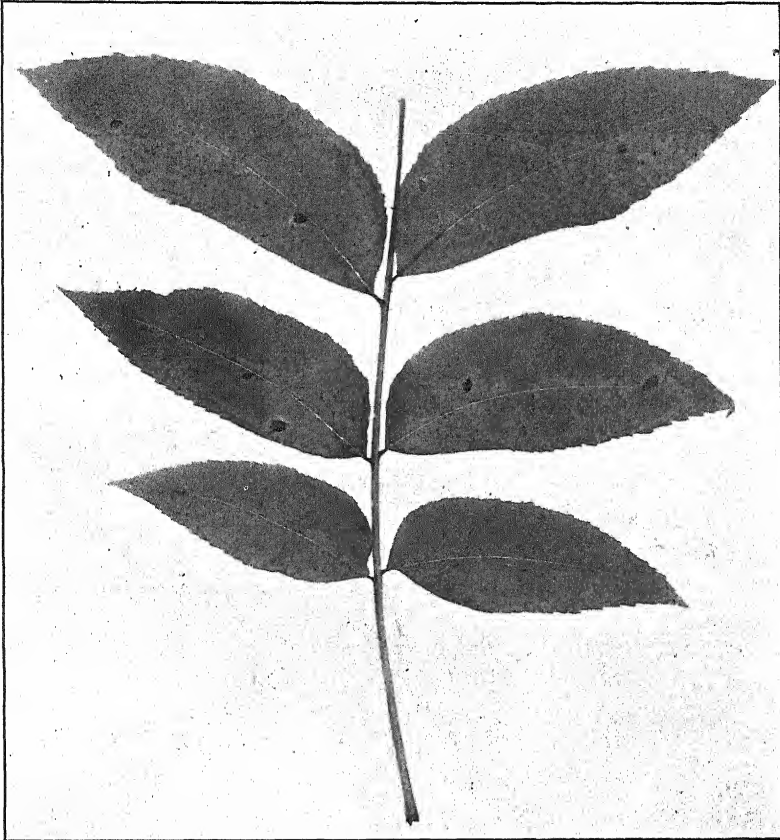


FIGURE 2.—Pecan leaf severely injured by the vein-spot fungus. The terminal leaflets have fallen and the petiole is discolored

bodies of the fungus are present. These fruiting bodies nearly always occur on the lower leaf surface and are usually confined to that portion of the lesion involving the vascular system.

Because of its striking similarity to pecan scab (*Cladosporium effusum* (Wint.) Demaree), vein spot has perhaps been confused with scab by growers and phytopathologists. Since scab spots no longer sporulating are black and are about the same shape and size as lesions of the vein-spot disease and since scab spots also appear on the leaf veins, it is difficult by casual examination to differentiate between scab and vein-spot lesions.

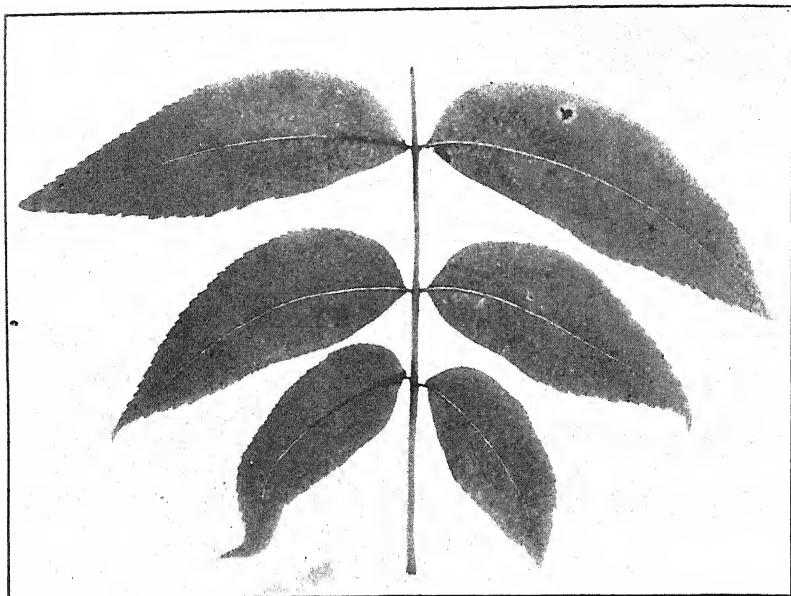


FIGURE 3.—Basal and upper leaflets of pecan leaf, showing characteristic lesions of the vein-spot disease. The terminal leaflets have fallen

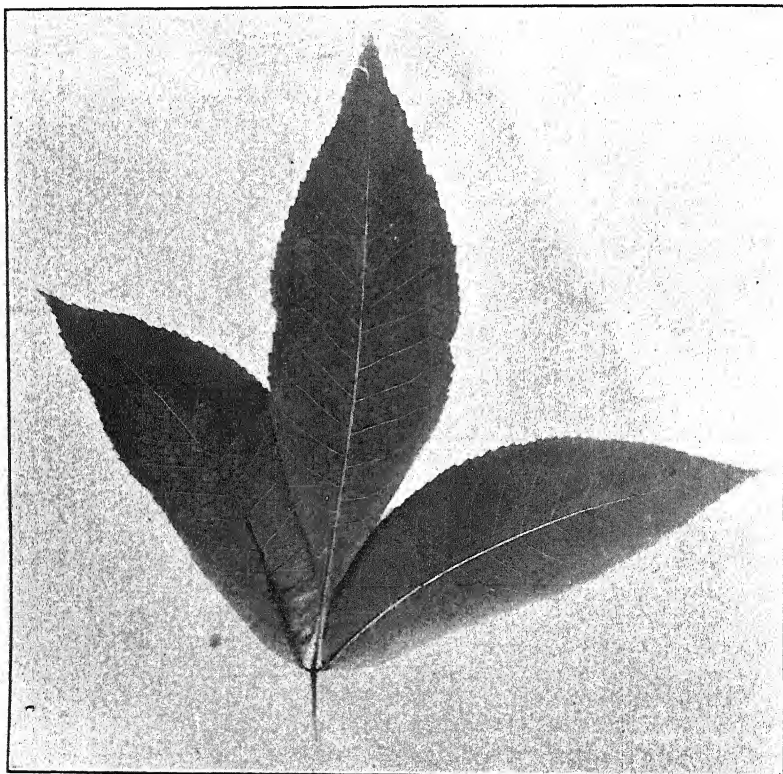


FIGURE 4.—Terminal leaflets attacked by the vein-spot fungus. Note the discoloration of the petiole and leaflet. The remainder of this leaf was normal



## THE CAUSAL FUNGUS

The fungus causing vein spot does not begin fruiting until late in the summer or in the fall. In the vicinity of Shreveport, La., a search for fruiting bodies on diseased leaves was made from June to September, 1930, but none was found earlier than about the first of September; they were not very abundant until about the middle of October.

## MORPHOLOGY

Just before the formation of fruiting bodies, which are considered as dimidiate pycnidia, the diseased areas on the underside of the leaf take on a grayish color. The fruiting bodies usually appear in these areas, either singly or in groups, and are difficult to see without some magnification. Usually they are intraepidermal or subepidermal (fig. 5), but occasionally they are found in the spongy parenchyma (fig. 6), and vary from  $35\mu$  to  $95\mu$  in diameter. They are embedded in the host tissues, apparently replacing the epidermal wall bordering on the spongy parenchyma and gradually destroying part of the latter. The covering of the fruiting body is irregular in outline and is composed of the somewhat thickened and blackened cuticle and epidermis. (Fig. 7.) This covering is occasionally torn in microtome sections, and several layers of tissue are found to be present. The coloring is probably due to some substance secreted by the fungus. Fungous tissue is no doubt present, but it is somewhat difficult to demonstrate it in the pycnidium. The base is made up of stromatic cells that penetrate the spongy parenchyma. In some instances layers four to five cells deep are formed.

## TECHNICAL DESCRIPTION

The fungus herein described is a new species with the following diagnosis:

*Leptothyrium nervisedum*, n. sp.

Pycnidia hypophyllis, depressis-pulvinatis,  $35\mu$ - $95\mu$  latis; conidiophoris simplicibus, septatis, rectis vel leniter curvatis,  $14\mu$ - $18\mu$  longis,  $2\mu$ - $4\mu$  latis; conidiis irregularibus interdum curvatis, oblongis-ovatis, hyalinis, continuis,  $8\mu$ - $13\mu$  longis,  $2\mu$ - $3\mu$  latis.

In foliis *Hicoriae pecan* (Marsh.) Britton, Louisiana, Mississippi, Arkansas, et Texas.

Pycnidia hypophyllous, depressed pulvinate,  $35\mu$ - $95\mu$  wide, conidiophores simple, septate, straight or slightly curved,  $14\mu$ - $18\mu$  by  $2\mu$ - $4\mu$ ; conidia irregular, sometimes curved, oblong to ovate, hyaline, nonseptate,  $8\mu$ - $13\mu$  by  $2\mu$ - $3\mu$ .

Lesions occur only on the vascular system of foliage of *Hicoria pecan* (Marsh.) Britton. The disease is found in Louisiana, Mississippi, Arkansas, and Texas.

When germination begins, a septum is usually formed dividing the conidium into almost even parts. Two germ tubes may come out from the conidium, one from each end (fig. 7), or the tubes may come out at either side.

## PHYSIOLOGY

The fungus causing the vein-spot disease of pecans was first cultured from mycelium early in the season. The cultures were made by placing the diseased host tissues in Petri dishes containing either cornmeal or pecan-leaf agar. Growth from the mycelium was very slow, the fungus requiring from two to three weeks to become macroscopic. Fruiting bodies were produced on the leaves in late summer, but the spores did not germinate until the latter part of October. When

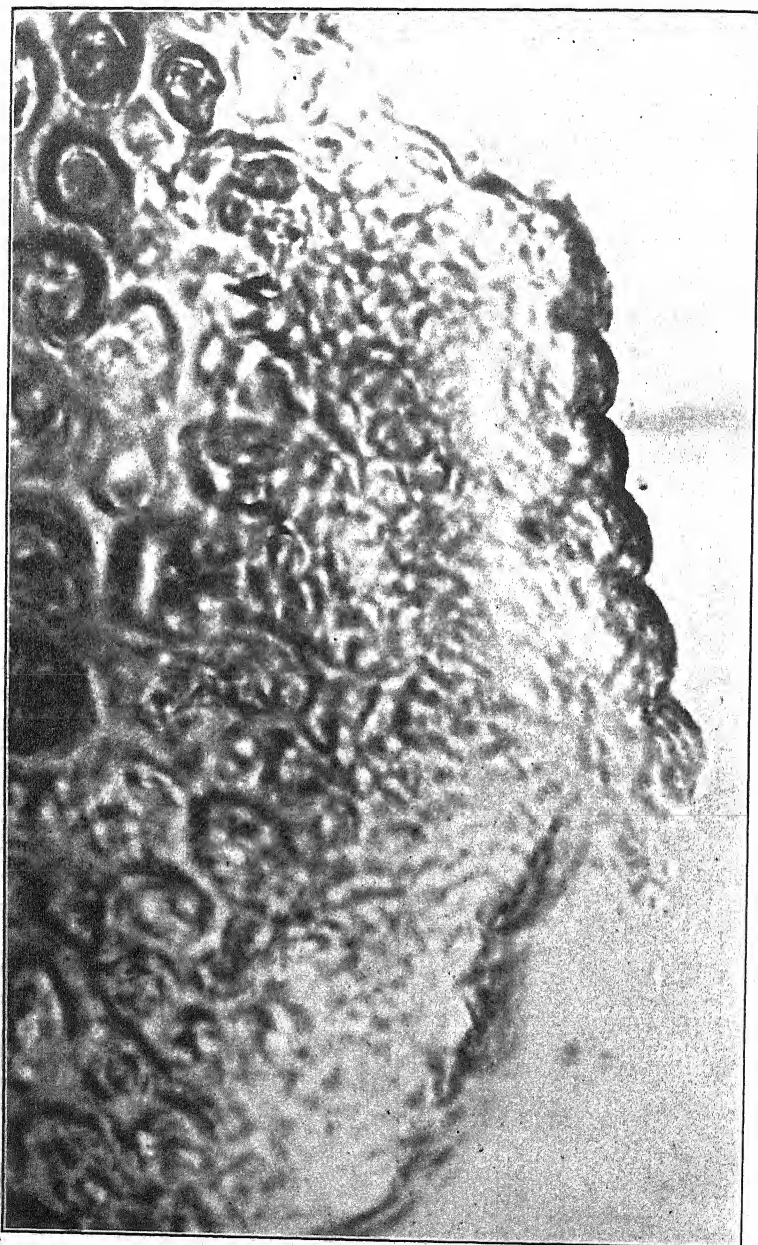


FIGURE 5.—Photomicrograph showing fruiting body of the vein-spot fungus produced above the spongy parenchyma of a pecan leaflet. The epidermal cells have been completely destroyed.  $\times 1,700$

placed on corn-meal or pecan-leaf agar, the spores sent out germ tubes from either end and sometimes from both ends. (Fig. 7.) Growth from the spores was likewise very slow at first, 15 days at 25° C. elapsing before monospore cultures became macroscopic.

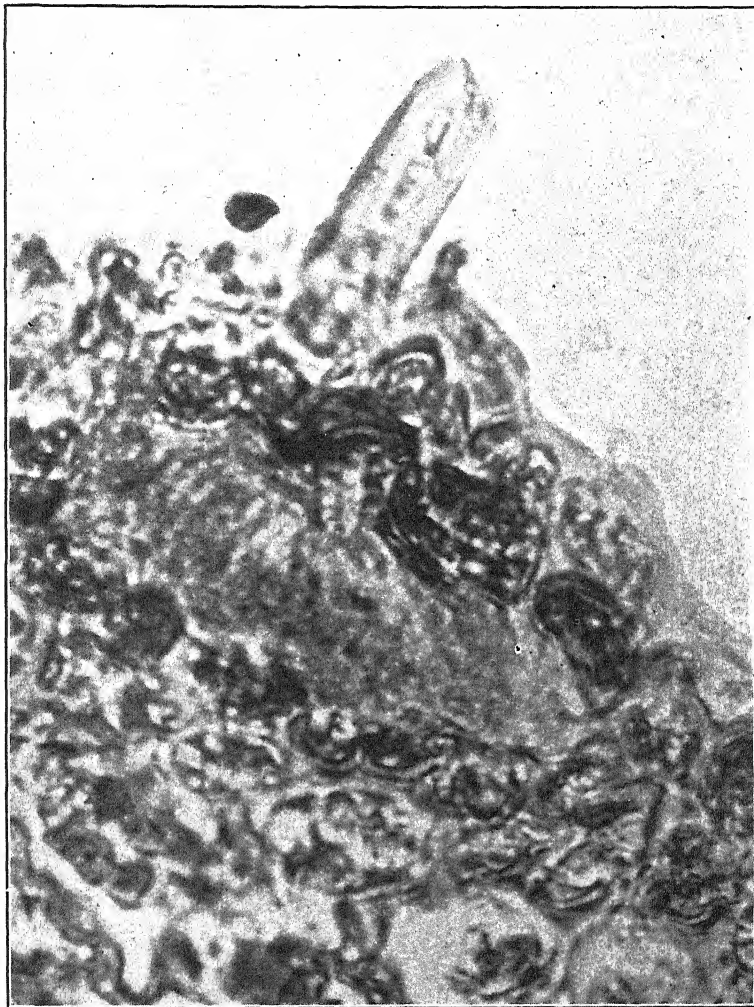


FIGURE 6.—Photomicrograph showing fruiting body of the vein-spot fungus produced in the spongy parenchyma of a pecan leaflet. The plant hair extends from the epidermal cells directly above the fruiting body.  $\times 1,700$

The fungus was grown on a variety of media, including nutrient agars, pecan-leaf and hickory-leaf agars, and vegetable plugs. A few pycnidia and spores were produced on corn-meal and on pecan-leaf agar, but the best growth, 9 cm in diameter, was made on potato plugs. In each instance the fungous growth consisted of a dark-brown to black tough stroma, mostly submerged and covered with short aerial hyphae.

## TEMPERATURE AND pH STUDIES

The vein-spot fungus was grown in an incubator<sup>4</sup> at constant temperatures ranging from 15° to 36° C. Poor growth occurred at 15°, whereas at the optimum temperature (26°) the fungus covered an area 7 cm in diameter. In all the compartments, except the one held at 36° where no growth occurred, the fungous growth was mostly submerged, brown in color, and covered with white or light-brown aerial hyphae. A few spores were produced in pycnidia at the optimum temperature. At all temperatures the fungus secreted a pigment that discolored the media. The color varied from light to dark brown, depending upon the amount of growth made by the fungus. When the fungus made optimum growth, the media became dark brown.

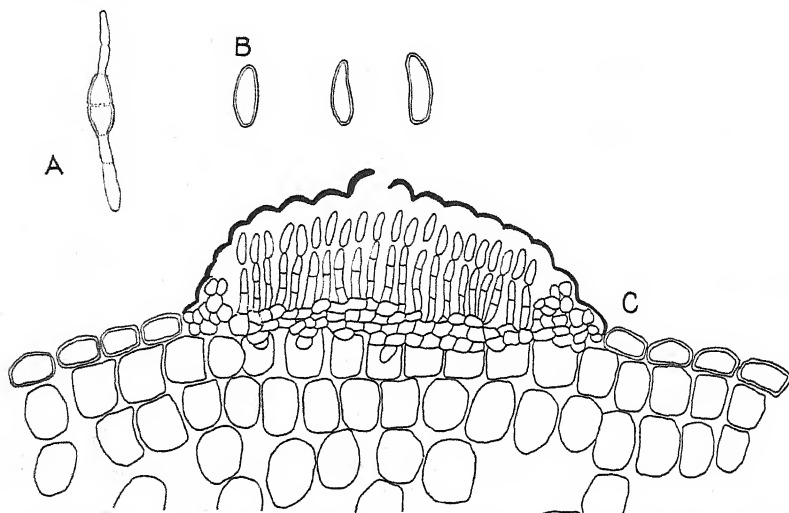


FIGURE 7.—The vein-spot fungus. A, Germinating conidium; drawing made after 30 hours.  $\times 500$ . B, Representative conidia.  $\times 500$ . C, Cross section of a leaf with the acervulus on the midrib.  $\times 250$

In order to study the influence of the hydrogen-ion content of the medium upon the behavior of the fungus, corn-meal agar was used in lots differing in acidity by 0.3 pH over a range of pH 4.0 to pH 8.6. The optimum growth (6.5 cm in diameter) was made at pH 5.9, whereas poor growth was made at pH 4.0 and pH 8.6. At all the degrees of hydrogen-ion concentration used, the appearance of the fungus was characteristic. The stroma was black, tough, mostly submerged, and covered with light to dark, short, aerial hyphae. The medium was also discolored, the discoloration depending upon the growth of the fungus, being dark brown at pH 5.9, the optimum growth, and lighter at pH 4.0 and pH 8.6. A few spores were produced in pycnidia at pH 6.6 and pH 6.9. Some of the spores were distorted, but most of them were normal.

## PATHOGENICITY

Pecan leaves of the Van Deman variety were inoculated with a heavy suspension of conidia taken from the diseased Van Deman

<sup>4</sup> The incubator consisted of 8 compartments each large enough to hold 2 dozen test tubes and 1 thermometer. The compartments were connected, with an ice-box at one end and a light bulb at the other. The incubator was so constructed and insulated that the temperature did not fluctuate more than 2°.

leaves collected in 1930. The inoculations were made as follows: Conidia were taken from diseased leaves and placed in a watch glass containing distilled water. Some were then placed on the leaves on or near the midrib or veins; others were placed between the veins. A little crayon was added to the inoculum to mark the inoculations. The leaves were then placed in glassine bags and kept there for 48 hours. The results are shown in Table 1.

TABLE 1.—*Results of inoculating leaves of the Van Deman variety of pecan with conidia of the vein-spot fungus*

Series No. <sup>a</sup>	Inoculations		Infections		Remarks
	Date	Number	Date of reading	Number	
1	Apr. 22.....	25	May 15.....	11	Spots, 1-3 mm long.
2	May 1.....	50	May 20.....	34	Do.
3	May 5.....	50	May 24.....	29	Do.
4	Aug. 9.....	25	Sept. 1.....	3	

<sup>a</sup> In series 1, 2, and 3, the inoculations were made on the veins; in series 4, the inoculations were made between the veins.

Uninoculated leaves held as checks remained free from the disease. The incubation period for the fungus appears to be between two and three weeks. In all instances infection took place on the vein or midrib, regardless of where the inoculations were made. In series 4 the infections took place on the veinlets, to which the spores were probably carried by insects or water from the place of inoculation.

In an effort to find the perfect stage of the fungus, infected leaves were collected in October, 1930, and placed outdoors in wire cages to overwinter. The leaves were examined at intervals of four weeks in order to ascertain whether any change in the life history was taking place; but no change was observed, and the conidia were found to be viable on May 1 of the following year. It is evident, therefore, that the fungus passes the winter in the conidial stage.

VARIETAL SUSCEPTIBILITY

Although vein spot has been found on both native and cultivated pecans, it is much more common on the cultivated varieties. In Louisiana the Van Deman variety is the most susceptible and the Frotscher variety is the next most susceptible; other varieties are attacked to about the same degree, depending somewhat on the locality. The writer is not familiar with many of the Texas varieties; the Kincaid variety, however, appeared to be attacked more severely than any of the others observed during the fall of 1931. Since both the Van Deman and the Frotscher varieties scab badly in southern Louisiana, it is sometimes difficult to distinguish between old scab lesions and vein-spot lesions. Scab causes more serious damage to the nuts than to the foliage; it is rarely serious enough to cause defoliation by itself, but probably acts in conjunction with other foliage diseases, especially vein spot, in defoliating the trees.

In the vicinity of Shreveport, La., the Van Deman is the only cultivated variety of pecan that has been attacked to any great extent, but since attacks by the fungus seem to be increasing on other varieties, vein spot may prove to be a serious disease within the next few years. There is some evidence that various strains of the vein-spot fungus exist. Other varieties of pecan which have been free from this disease but which grow near trees of the Van Deman variety

that have been prematurely defoliated for several years are also becoming diseased by the vein-spot fungus. It may be that the fungus is gradually adapting itself to other varieties. For example, in one orchard where Schley trees are growing near Van Deman trees that have been prematurely defoliated for the past two years, the writer was unable to find any signs of the disease on the Schley during the season of 1930, but in 1931 a few of the Schley leaflets were infected. There are instances of other varieties that have become infected in a similar manner.

Since the disease is present near the Gulf coast in Louisiana and Mississippi, where the annual rainfall is about 60 inches, and is also present in the central part of Texas, where the annual rainfall is only about 25 inches, it would seem that humidity is a negligible factor in its development. Probably vein spot attacked the native trees of Texas first and is now spreading eastward.

#### CONTROL

Although no experiments have been conducted with the object of controlling vein spot, the following observations have been made in experiments conducted for the control of pecan scab. In orchards where the growers dusted for control of scab on the Van Deman variety in 1929, using 4 applications of 20-80 monohydrated copper sulphate and lime dust, the vein-spot fungus was held in check, whereas on trees that were not dusted this fungus caused defoliation by September 15. During the season of 1931, in experiments conducted for scab control on the Frotcher variety in southern Louisiana, 4 applications of a dust composed of copper sulphate monohydrate (20 per cent) and hydrated lime (80 per cent), alternating with 3 applications of Bordeaux mixture composed of 3 pounds of copper sulphate (bluestone) and 4 pounds of lime in 50 gallons of water, resulted in about 90 per cent vein-spot control. The check trees were defoliated by vein spot, together with *Cercospora fusca* F. V. Rand. In each of the experiments just described, the first application of dust or spray was made about the middle of May, and the last was made about August 1.

#### SUMMARY

Vein spot, a foliage disease of the pecan (*Hicoria pecan* (Marsh.) Britton) caused by *Leptothyrium nervisedum*, here described as new, has been collected by the writer in Louisiana, Arkansas, Mississippi, and Texas.

The fungus attacks the vascular system of the leaf, thereby suggesting the name "vein spot." Heavy infection results in severe injury to the foliage or in premature defoliation.

A varietal susceptibility to the fungus appears to exist, especially in Louisiana. The Van Deman is the most susceptible variety, and the Frotcher is the next most susceptible.

Since vein spot is found both in regions of high and of low rainfall, humidity is not considered to be a factor in the development of the disease.

No experiments for the control of vein spot have been conducted. However, very good results in the control of this disease were observed in plots where experiments were in progress for controlling scab by spraying three times with 3-4-50 Bordeaux mixture, and alternating with four 20-80 monohydrated copper sulphate and lime dust treatments.

## PLASTER MOLDS OCCURRING IN BEDS OF THE CULTIVATED MUSHROOM<sup>1</sup>

By VERA K. CHARLES, *Associate Pathologist*, and EDMUND B. LAMBERT, *Associate Pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture*<sup>2</sup>

### INTRODUCTION

Among the numerous fungi that may be found in the compost of mushroom beds in the United States, two are often conspicuous. One develops over the surface of the manure in patches that are white in the early stages of growth, turning cinnamon-brown after a few days. The other fungus grows deep into the beds, forming a white powdery mass that may become a pinkish gray but never brown. These molds are responsible for considerable economic loss, and most experienced growers are familiar with their gross appearance. A great deal of confusion, however, has arisen from the use of different common names by different growers and from the fact that the relation of these fungi to described species has not been clearly defined. In an attempt to clear up this confusion the writers have isolated and critically examined cultures of both fungi from several localities. The present paper reports data on their distribution, variability, and probable relation to species described in foreign countries. In the following discussion the subsurface mold will be designated "white plaster mold" and the surface mold "brown plaster mold".

### WHITE PLASTER MOLD

White plaster mold is the more injurious of the two fungi under consideration. It penetrates deeply into the beds and gives the compost the appearance of having been dusted with flour. It greatly retards or completely inhibits the growth of mushroom spawn, so that its presence in the beds frequently results in the total failure of the crop.

The occurrence of plaster mold in the United States was first brought to the attention of the Department of Agriculture in 1897, when material was received from San Rafael, Calif., with the information that the spawn had made a certain amount of progress but had suddenly been checked. The second report of the occurrence of this disease in the United States was received in 1920, this report also coming from California. Two years later the same grower reported that a loss of over \$100,000 had been suffered by California growers, and his personal loss was estimated at from \$20,000 to

<sup>1</sup> Received for publication Feb. 23, 1933; issued July, 1933.

<sup>2</sup> Acknowledgment is made of a culture of white plaster mold from Dr. Louise Dosdall, University of Minnesota, and of two cultures from Dr. W. M. Ware, of England, in 1930.



\$30,000 during a period of 4 years. Since then the writers have observed similar failures and have identified and isolated the fungus from collections made in Pennsylvania, Minnesota, California, Idaho, Illinois, and New York.

White plaster mold is also a serious pest in France, Italy, and England. Detailed accounts of extreme losses from this fungus are given by Costantin and Matruchot (5)<sup>3</sup> for France, and Cuboni and Megliola (6) for Italy. Recently Ware (14, p. 19-20) in England also mentioned it as a competitive invader in mushroom beds.

#### IDENTITY

The various collections of white plaster mold made by the writers in four different States and from many different beds agree very well with the original description of *Monilia fimicola* by Costantin and Matruchot (5, p. 292), who found it causing a disease of mushroom spawn in 1894. Because of the plasterlike character of this fungus they referred to it as "le plâtre".<sup>4</sup> Their description is as follows:

Mycelium colorless, septate, little branched,  $2\mu$ - $5\mu$  in diameter. In pure culture the sporiferous filaments are first in groups of 4 or 5, and the groups are separated from each other by a long portion of sterile mycelium.

The development is described as follows:

The sporiferous filament is at first simple and fruits at its extremity, soon branching irregularly, each branch producing a chain of many spores. The fruiting filaments vary from  $50\mu$  to  $100\mu$ , are cylindrical at the base and the same size as the mycelium, diminishing gradually toward the extremity.

Unfortunately, in this description the size and shape of the spores were omitted. However, the shape of the spores and their size in relation to the mycelium are shown clearly in the drawings (5, pl. 13, figs. 9-13). In 1906 Saccardo (11, p. 503) described the spores as elliptical with obtuse ends and  $6.5\mu$ - $8\mu$  by  $4.5\mu$ - $5.3\mu$  in size. The inference is that these measurements were estimated from the magnification of the drawings. They agree with the measurements of spores in the writers' cultures. Figure 1 shows spores from a culture of *Monilia fimicola*. The U-shaped dichotomous branching of the sporophores as shown in the drawings of Costantin and Matruchot was also found to be quite characteristic of the writers' cultures. Figure 2 shows U-shaped branching of the mycelium. Figure 3 is from a culture of *M. fimicola* showing mycelium and spores.

In 1903 the Italian investigators Cuboni and Megliola (6) published an account of this disease in Italy. Their observations led them to the conclusion that the Italian fungus was identical with *Monilia fimicola*; but because the mycelium was limited to the substrata, and because the spores were extremely small, they referred it to the genus *Oospora*, designating it *Oospora fimicola* (Cost. and Matr.) Cub. and Megl. The two descriptions of this fungus by the French and the Italian authors, respectively, are very similar, the main difference being in the shape of the spores. Cuboni and Megliola describe them as globose, whereas those in the drawings of Costantin and Matruchot (5) are ovoid. In the course of this study the writers

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 1097.

<sup>4</sup> It should be noted here that Costantin (4) first applied the name "plâtre" to a very different fungus, *Verticillium infestans*, but later referred to *Verticillium* as the "faux plâtre" of the gardeners.



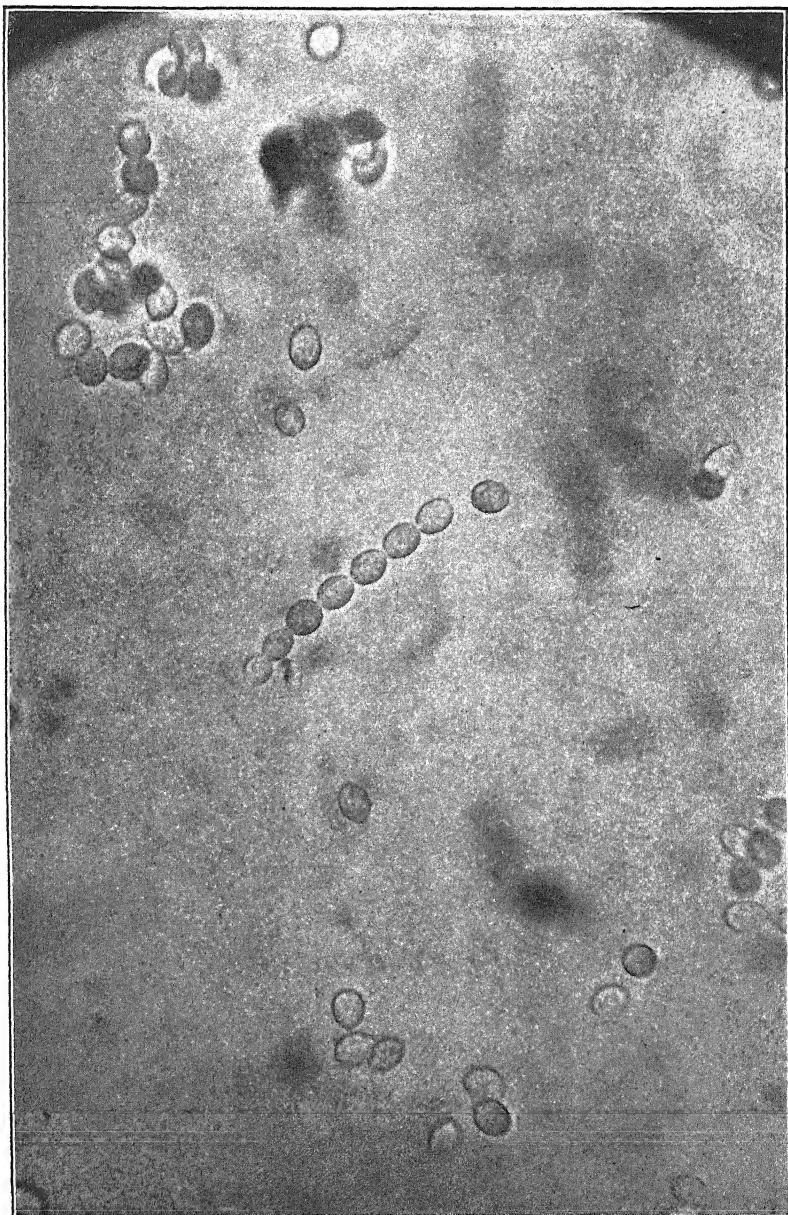


FIGURE 1.—Spores from culture of *Monilia fimicola*.  $\times 800$

examined a specimen from Cuboni distributed by D. Saccardo<sup>5</sup> and found only a few globose spores, the majority being oval. It is noteworthy in this connection that the spores in the Cuboni specimen also correspond in size and shape with the spores from the writers' cultures. Whether this species is to be considered as belonging to the genus *Monilia* or to the genus *Oospora* is largely a matter of interpretation. Since sterile hyphae are plentiful on ordinary media, the writers prefer the older name *Monilia fimicola* Cost. and Matr.

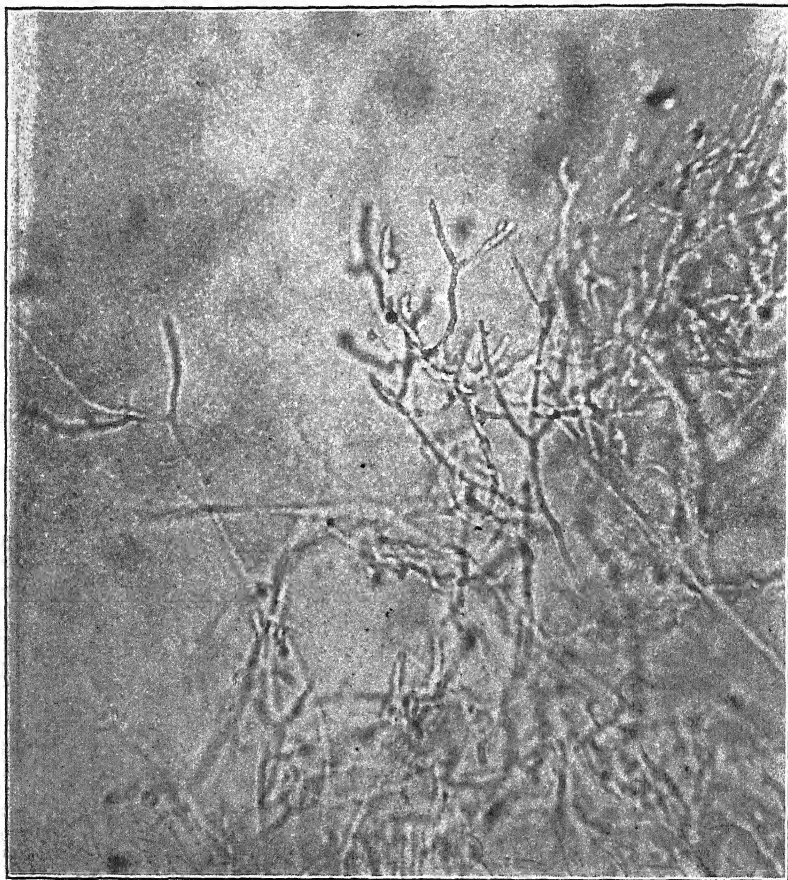


FIGURE 2.—U-shaped branching of the mycelium of *Monilia fimicola*.  $\times 380$

In 1930 W. M. Ware sent the writers two cultures of *Monilia fimicola* which he had collected in England in 1929. One was isolated from a sample of dung from a mushroom bed at Bradford-on-Avon, Wiltshire, and the other was from a fragment of brick spawn at Lewes, Sussex. Both of these cultures were similar in shape and size of spores and general cultural characters to those isolated by the writers in the United States.

<sup>5</sup> *Mycotheca italica* Mucedinaceae, 1572, *Oospora fimicola* (Cost. et Matr.) Cub. et Megliola, Albano (Roma)—in palea putri et in fimo equino—valde noxia mycelio Agarici campestris—November 1903, Prof. G. Cuboni.

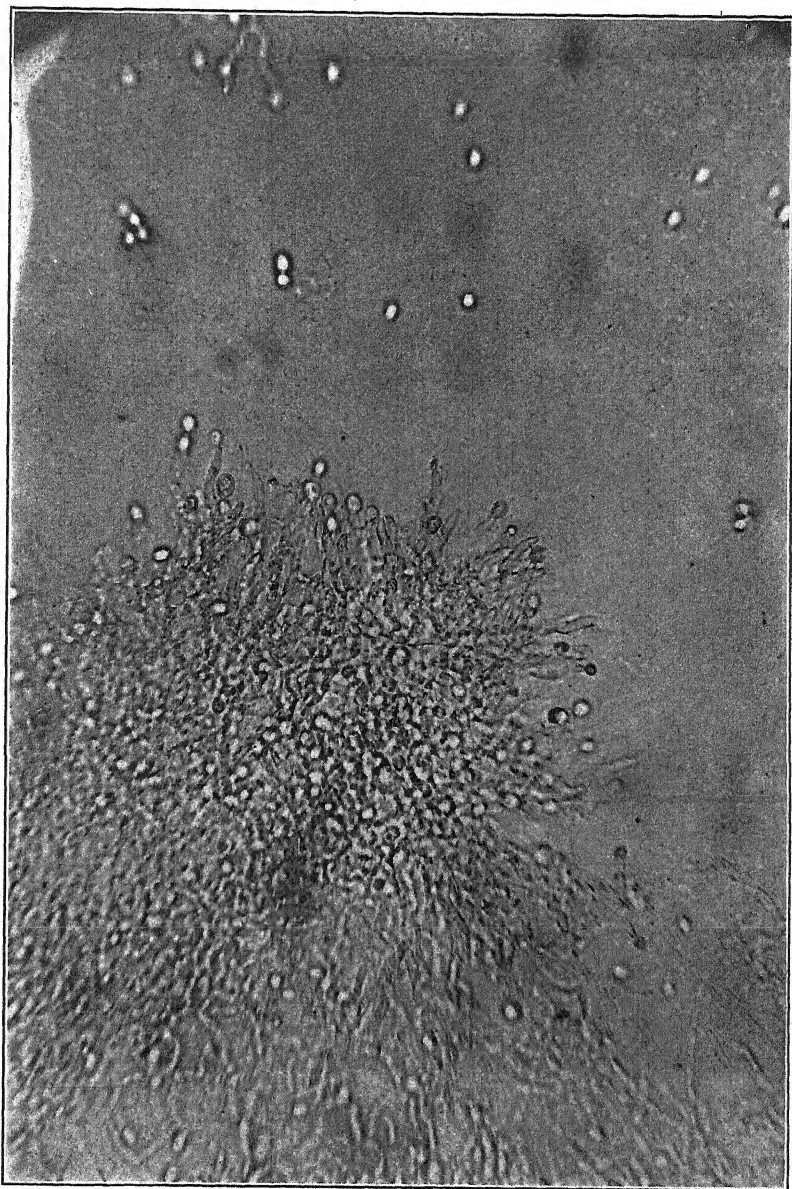


FIGURE 3.—Culture of *Monilia fimicola* showing mycelium and spores.  $\times 380$

## PHYSIOLOGIC SPECIALIZATION AND MUTATION

Despite the rather constant morphology of spores and sporophores in cultures isolated from different collections of *Monilia fimicola*, there were often distinct differences in the cultural characters of strains originating from different collections when grown under identical conditions. In some cases the pale pinkish-buff color typical of the fungus mat and the coloring of the medium were entirely absent. These differences were especially noticeable when the cultures were grown in triplicate Erlenmeyer flasks on potato-dextrose agar. In the writers' opinion the differences were sufficient in four cases to indicate distinct physiologic forms, as this term is used by Stakman and his coworkers (2, 12, 13).

It is also of interest to note that sectors arose in several of the cultures when grown in flasks. These sectors were oftentimes quite

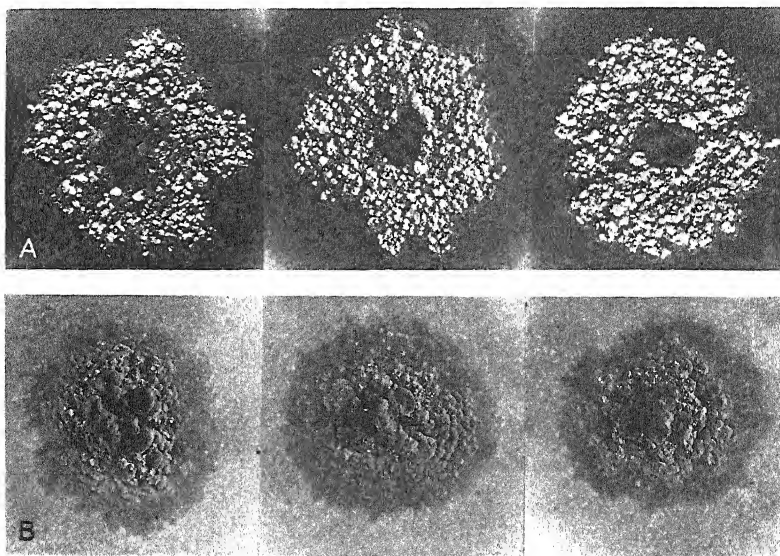


FIGURE 4.—Physiologic forms and sectoring in *Monilia fimicola*: A, Cultures of strain from California; B, from Idaho, middle culture of latter showing sector emerging.  $\times 3\%$ .

distinct from the parent cultures. For example, some of the sectors were ivory-yellow and waxy, while the fungus mat of the parent cultures was pale pinkish-buff and diffuse powdery. Subcultures from these sectors had the same general cultural characters as the sectors. In monosporous cultures sectors arose which were as distinct as those appearing in mass cultures. The writers interpret these sectors as arising from mutations, as this term is used by Stakman, Christensen, et al. (2, 12, 13). The cultures shown in figure 4 are triplicate subcultures from monosporous cultures of two strains of *Monilia fimicola* grown under identical conditions on Filco potato-dextrose agar in 250-cc Erlenmeyer flasks. The strain shown in the upper row was collected in a mushroom bed near San Francisco, Calif., and that shown in the lower row was collected near Boise, Idaho. There is an evident difference in the appearance of the

fungus mat and the coloring of the medium produced by the two strains. A sector may be seen emerging from the central culture of the Idaho strain.

#### BROWN PLASTER MOLD

Brown plaster mold is popularly so called because of the cinnamon-brown color which characterizes it at maturity. It has been found in varying amounts in almost all commercial mushroom houses visited by the writers in the eastern part of the United States. It has been known to commercial mushroom growers for some years and was first observed by the senior writer in Pennsylvania and New York in 1923. It is typically a surface grower both when it occurs in nature and when grown artificially on culture media. Over the surface of the beds it forms patches that are usually from 6 to 15 inches in diameter. These patches, at first white and plasterlike, soon change to tan and finally to cinnamon-brown, becoming less conspicuous as they mature. In most cases similar patches may be observed under the side boards. Sometimes several patches will coalesce to form a continuous coating over the surface of the compost. When the casing soil is applied to infested beds the fungus reappears after a short delay over the surface of the casing soil. Mushroom mycelium seems to experience considerable difficulty in penetrating these spots. As a result the production of sporophores is retarded within infested areas, and the yield is noticeably reduced.

#### IDENTITY

If the white patches over the surface of the beds are examined with a hand lens they are seen to consist of numerous spherical bodies. Occasionally these bodies are elongated by the fusion of two. In a few instances there may be a prolongation suggestive of a neck. In size they range from  $80\mu$  to  $110\mu$  in diameter. In the early stages they consist of mostly hexagonal isodiametric cells. As they develop, the cells of the outer layer become somewhat flattened and pigmented.

Hein (8, 9) isolated cultures of this fungus and found it ideal material for study of concentric rings in culture and the production of tetrakaidecahedron in pseudoparenchyma. He considers the spherical bodies sclerotia and gives excellent illustrations of their structure and cultural characters but does not attempt to identify the fungus with any known species.

The authors have found the so-called sclerotia collected from mushroom beds to be practically identical with those in a specimen of *Myriococcum praecox*<sup>6</sup> in the Mycological Collections of the Bureau of Plant Industry. Two other specimens of this fungus were also examined, one in the Mycological Collections<sup>7</sup> of the Bureau of Plant Industry and a second specimen from Denmark, determined by Rostrup. These two collections also correspond with the writers' collections. The species was described by Fries (?) in 1823 as the type of *Myriococcum*, a poorly described and little understood fungus.

The systematic position of *Myriococcum* is very uncertain, and by most modern authorities *Myriococcum* is placed among genera dubia in the Perisporiales. It was placed by Fries in the class Gasteromy-

<sup>6</sup> FRIES, E. *SCLEROMYCETI SUECIAE*, Dec. 7, No. 70.

<sup>7</sup> Collected by A. B. Langlois, No. 914, on decaying wood in Louisiana.



cetes, order Angiogastres, suborder Nidulariaceae, and by Corda (3) in the family Physarei, section Eurotiaceae. In 1842 Corda (3) published the results of his observations and expressed doubt as to whether or not the interior of the fungus body was chambered or hollow, but was inclined to the latter belief, as no fragment of an interior wall could be found. He stated that the spore mass filled the interior completely, and he described the spores as elongate, many sided, nearly wedge-shaped, white, and transparent. From numerous examinations it would appear that what have been called spores are in reality the inner cells which are less compressed and therefore not as uniformly hexagonal as the outer cells.

Attention may also be called to the close resemblance of *Sclerotium eurotioides* Lib. to the brown plaster mold. *S. eurotioides* was described in 1832, the description on the specimen label reading as follows: "Congestum tectum, minutissimum, globosum, leve, stramineum, demum fulvum, 100 $\mu$  diameter; villo mucido demum evanescenti insidens."<sup>8</sup> A Libert specimen of this fungus in the Mycological Collections of the Bureau of Plant Industry appears identical in size and structure. The color is slightly lighter in the Libert material, but the difference is slight and may be due to age or the difference in the substratum. The writers are inclined to consider the spherical bodies either as abortive perithecia or the bulbils of a Hymenomycete.

#### DISCUSSION

Evidence has been presented which shows that the white and brown plaster molds are distinct and referable, respectively, to *Monilia fimicola* Cos. and Matr., and *Myriococcum praecox* Fries. Comparative studies of the cultural characters of eight different collections of *Monilia fimicola* from different localities in the United States and England bring out the fact that biotypes exist in this species which sometimes have quite different cultural characters under identical conditions and presumably differ likewise in their general physiology. Thus caution is necessary in generalizing from physiological data obtained for this species, such as thermal death curves, in relation to control practice. In one collection an unidentified *Monilia* was isolated which has pointed spores averaging 6 $\mu$ -6.5 $\mu$  by 3 $\mu$ -3.5 $\mu$ , but since *M. fimicola* was isolated from the same collection the significance of the unidentified fungus is problematic.

The brown plaster mold (*Myriococcum praecox*) has never been described in connection with European mushroom culture, and it is evident that the white plaster mold (*Monilia fimicola*) is identical with "le plâtre" of France and the "plaster mould" of England. For this reason the terms "brown plaster mold" and "white plaster mold" are preferred by the writers to the terms "plaster mold" (for the *Myriococcum*) and "flour mold" (for the *Monilia*), which are sometimes used (1). These terms are perhaps as descriptive as white and brown plaster mold under domestic conditions, but they may lead to confusion when comparisons are made between domestic and foreign conditions.

To avoid confusion of the plaster molds with other species of *Monilia* encountered in connection with mushroom culture, mention should be made of still another species of *Monilia*, one belonging to

<sup>8</sup> LIBERT, M. A. PLANTAE CRYPTOAMICAE ARDUENNAE, Cent. 2 (1832), No. 138.

the *M. sitophila* group. This species has come to the attention of the writers intermittently over a long period of years as a contaminant in the manufacture of spawn. A recent French paper by Moruzi (10) dealing with this fungus is entitled "Sur une Maladie du Champignon de Couche Causée par un *Monilia*." This title would seem to be rather misleading, as the fungus was only encountered in the manufacture of spawn. Furthermore, Moruzi's paper deals with the cytology of the organism rather than with its role as an invader in mushroom beds. The writers' observations lead them to believe that *M. sitophila* cannot successfully compete with the microbial flora in well-composted manure and that it is seldom, if ever, found on the beds with the plaster molds.

#### SUMMARY

Serious losses are suffered by mushroom growers in the United States through the presence in mushroom beds of certain fungi popularly known as plaster molds. The most dangerous of these fungi is the white plaster mold found to be identical with "le plâtre" of France and described by Costantin and Matruchot in 1894 as *Monilia fimicola*. This species has been collected in California, Idaho, Illinois, Minnesota, Pennsylvania, and New York; and while the collections are morphologically identical, there are physiologic forms in the species, and sectors frequently appear in flask cultures. *M. fimicola* is also recognized as a serious menace to successful mushroom growing in England and Italy.

The second fungus, brown plaster mold, although commonly present in mushroom beds in the United States, has not been described in connection with mushroom culture in Europe. It is here recognized as identical with *Myriococcum praecox*, a fungus described by Fries in 1823.

Attention is also called to the occurrence of *Monilia sitophila*, which should not be confused with the plaster molds. It often appears as a troublesome contaminant in the manufacture of spawn both in the United States and Europe, but is seldom, if ever, found in composted manure with the plaster molds.

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## A PHOTOMICROGRAPHIC STUDY OF GELATINIZED WHEAT STARCH<sup>1</sup>

By SYBIL WOODRUFF, *Associate Chief in Foods*, and LUCILLE RANKIN WEBBER, *Graduate Student, Illinois Agricultural Experiment Station*

### INTRODUCTION

A rigid paste of starch and water is responsible to an important degree for the physical properties of baked products made with wheat flour. Starch constitutes about 55 per cent of the weight of the starch-water fraction of bread dough and about 35 to 40 per cent of that of muffin and cake batters. The ratio of starch to its own combined water is even higher than these figures indicate because not all the water present in a baked flour mixture is free to go to starch but is associated with proteins and other constituents; nor is all the starch in bread necessarily as completely hydrated as it was in the gels studied here. Even so, such amounts of starch are relatively great as can be seen from the fact that a cooked starch paste containing only 5 per cent starch will upon cooling set to a tender gel which retains the shape of a mold.

The shapely mass produced by heating together starch and water will be called in this paper a gel, and the possibility that its internal structure may be unlike that of gels produced by particles known to be of colloidal dimensions will be ignored. Starch gels, especially those of about 5 per cent concentration, have properties in common with gels of gelatin and pectin; they appear quite homogeneous, can be cut to leave a straight-edged surface, and may show syneresis upon standing. These characteristics all play a significant rôle in starchy food preparations.

The heating of starch in the presence of water brings about changes which have been rather unanimously called "gelatinization," though different workers have interpreted the term to refer to changes with respect, in turn, to microscopic appearance of the granules, translucency of suspensions, and viscosity of fairly dilute pastes. Changes in such factors occur over a rather wide range in temperature. When gelatinization has proceeded to a certain point, a suspension will often set to the gel form already mentioned as a desirable one in many food preparations.

It was with the purpose of relating the factors of concentration, temperature of heating, and microscopic changes in the granules to the ability of a suspension to assume form as a gel that this work was undertaken. Records on hard and soft wheat starches were obtained in the form of photomicrographs showing the altered gran-

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<sup>1</sup> Received for publication Aug. 5, 1932, issued July, 1933.

ules at temperatures such as were found capable of producing moldable gels. Changes of starch in physical state are of interest from the standpoint of their connection with palatability and adaptability to storage and have no significant bearing, according to the literature, upon digestibility.

## REVIEW OF THE LITERATURE

As a source of information concerning the microscopic characteristics of raw starch granules of almost every variety, Reichert's compilation (21)<sup>2</sup> of photomicrographs is most complete. Starches are shown under both ordinary and polarized light. Fall (7) has more recently published photomicrographs of a few raw specimens. As for starches which have been altered by heating in water, the literature contains descriptions but not photomicrographs. Alsberg (1) has in part based his explanation of the behavior of cooked pastes, an explanation now generally accepted, on the fact that microscopic observation shows that most of the granules remain whole though very much swollen. For this reason, he says an ordinary paste is a suspension, and not a colloidal solution as earlier writers have said. The viscosity of the paste, dependent upon the jostling of the swollen granules, can be much reduced by injury to the outer covering of the grain, though disruption of all granules is difficult to attain even by long grinding.

The crystallin structure of starch granules has been studied by Katz and his associates (13, 14, 15, 16, 17, 18) who, with X-ray spectrograms, have found evidence of three forms—original, gelatinized, and retrograded starch. The spectra pointed to a structure within the granule which has not yet been revealed by the microscope and indicated that physicochemical changes occur in the granule during gelatinization. In microscopic appearance, gelatinized wheat-starch granules appeared to Katz (12) as swollen vesicles, many times their original size and showing in the center a cavity due to stronger swelling in the tangential than in the radial direction. He distinguished a first and a second degree of gelatinization encountered respectively in the changes of bread baking and in gelatinization of starch to a paste in the presence of a large volume of water. The same phenomenon of change in X-ray spectrogram was observed in both first and second degree gelatinization.

A number of investigators (3, 6, 8, 21) have pointed out the relation between disappearance of birefringence and gelatinization temperatures of different starches. The concentration of the suspension examined by Reichert for loss of double refraction was not given, but he reported that anisotropy left wheat starch granules between 65° and 67.5° C. Alsberg (2) found no individual birefringent granules in bread crumb though birefringent masses could be seen. In wheat starch, the largest granules swelled and lost their anisotropy at lower temperatures than did the small ones. Alsberg and Rask (3) concluded as a result of viscosity measurements that gelatinization occurs over a temperature range of 25° to 30°. There was no significant change in the viscosity of 4.5 to 5.0 per cent wheat-starch pastes up to 65°, but a gradual increase occurred from 68° to 95°.

<sup>2</sup> Reference is made by number (*italic*) to Literature Cited, p. 1106.

Ripperton (22) devised a means of measuring the swelling power of starch in dilute suspensions and concluded from his work on potato and canna that swell and viscosity are not directly proportional. Monovalent cations increased swell and viscosity while calcium depressed them. Chapman and Buchanan (5) pointed out the influence of certain salts in preventing or retarding syneresis by forming a firmer gel structure. Woodruff and Nicoli (23) showed that large amounts of sugar destroyed the gel-forming power of 5 per cent starch suspensions. Hall (11) found that wheat starch heated for 1 hour at 100° C. in the presence of one-half its weight of water remained as a white powder though each individual granule had swollen. When heated under these conditions but with an equal weight of water, it became transparent, opalescent, and extremely swollen. Woodruff and Nicoli (23) were able to obtain a moldable gel at 90° when 5 per cent suspensions of a commercial wheat starch were handled.

Unusually great variation in the size of wheat-starch granules is a characteristic which several investigators have attempted to correlate with physical behavior. Furry (9) found that an average of 50 wheat-starch granules swelled to about 5 times their original size. This increase was higher than that for any other starch measured, except potato. Buchanan and Naudain (4) thought that superior bread-making qualities of hard-wheat flours might be correlated in some way with the high percentage of small starch grains found in hard-wheat flours as compared with soft ones. Rask and Alsberg (20) also found variation in the viscosity of pastes made of hard and soft wheat starches and thought that this factor might possibly be concerned with baking qualities. Grewe and Bailey (10), however, found no correlation between baking tests, diastatic activity or heat of imbibition, and the size of starch granules. Ripperton (22) said that neither size of granules of potato and canna starches nor the number of unbroken ones present could account for the differences in viscosity that he observed.

The ability of a starch paste to form a gel varies with the source of starch (23) and, it is believed by the authors, with the conditions of heating. Such variable factors as were studied here might easily occur in the common methods of processing used in the factory or home.

## EXPERIMENTAL METHODS

### PREPARATION OF WHEAT STARCH

Starch was specially prepared from flours which had been milled for another experiment-station project from single varieties of wheat. One was Fulhio, a soft red winter wheat which had been milled in a commercial soft-wheat mill. The other was a hard winter Turkey wheat milled in a hard-wheat laboratory mill. The method of separating the starch was that recommended by Alsberg and Rask except that during the washing of the starch from the gluten the starch suspension was passed through silk bolting cloth of 125 meshes per lineal inch. The starch was air-dried for use and was free from extraneous matter as shown by the following analyses: For hard-wheat starch, moisture 10.65 per cent, ash 0.09 per cent, starch (by Rask's (19) method) 88.92 per cent; for soft-wheat starch, moisture 10.60 per cent, ash 0.08 per cent, starch 89.39 per cent.

Starch-water suspensions containing 5, 10, and 50 per cent starch by weight were used because of the likely occurrence of such concentrations in common starchy food preparations.

#### METHOD OF GELATINIZING THE STARCH

One hundred grams of a combination of starch and water were mixed in 200-cc glass jars whose screw caps carried holes for a thermometer and a sampling pipette. The jars were immersed in a water bath almost to the metal cap. The suspensions were stirred with a glass rod at 15-minute intervals while they were being heated and until they reached 80° C. after which they showed no tendency to settle. The 50 per cent suspensions were stirred just before 60° after which they were too stiff for further manipulation. At specified temperatures, samples of the 5 and 10 per cent suspensions were removed by pipettes to fill small crucibles which served as molds. Samples were scooped out of the center of the 50 per cent paste with a hemispherical metal spoon. The gels were turned out onto watch crystals after 24 hours and photographed. Samples were taken for microscopic examination at the same time that they were removed for gel purposes.

A large water bath was electrically heated and stirred and was so adjusted that its temperature rose from 50° to 93° C. in 45 minutes. When the bath was already at a temperature of 93° before the jars were entered, the temperature of the suspensions reached 90° in 20 minutes. Almost continuous stirring was required with this more rapid rate of heating. A thermostatic control made it possible to hold the temperature at 90° for as long a time as desired. This large electric bath attained no higher temperature than 99.5° and permitted a maximum temperature inside the jar of only 95° to 96°.

Preliminary work had indicated that the physical character of the resulting gel depended somewhat upon the rate of heating as well as upon the final temperature reached. In view of this fact, suspensions were heated more rapidly than the electric bath permitted by immersing conical flasks containing them in a large beaker of boiling water, and still more rapidly in another case by heating the contents of the flask to boiling over a direct flame. The flask was removed as soon as the contents boiled. The temperature was found to be 99° to 100° C.

In certain instances, all cold water was used in making the 5 per cent suspension; in others, the starch for 100 g of paste was moistened with 10 cc of cold water, and the remainder of the water was added near its boiling point from a pipette. The contents were mixed by being swirled, and by the time all the hot water was added the temperature had fallen to about 70° C. In some cases, hot water was used for the first mixing even though the suspension was later to be heated in a bath whose temperature at the outset was only little above room temperature. The 10 and 50 per cent suspensions were mixed by the first method and were heated slowly in the electric bath from room temperature to the one indicated.

#### MICROSCOPIC TECHNIC

Samples of starch paste were removed by pipette from the jar at the desired temperatures and before the paste had solidified; one wire loop of suspension was transferred to two loops of water on a micro-

scope slide. In the case of the stiff 50 per cent suspensions, a pinhead portion taken from the center of a freshly opened lump was used for mounting. By a gentle upward and downward motion of the loop, the starch and water were mixed and spread. A cover glass was adjusted without pressure and sealed at once to the slide with a preparation of beeswax and paraffin. This kept the granules moist

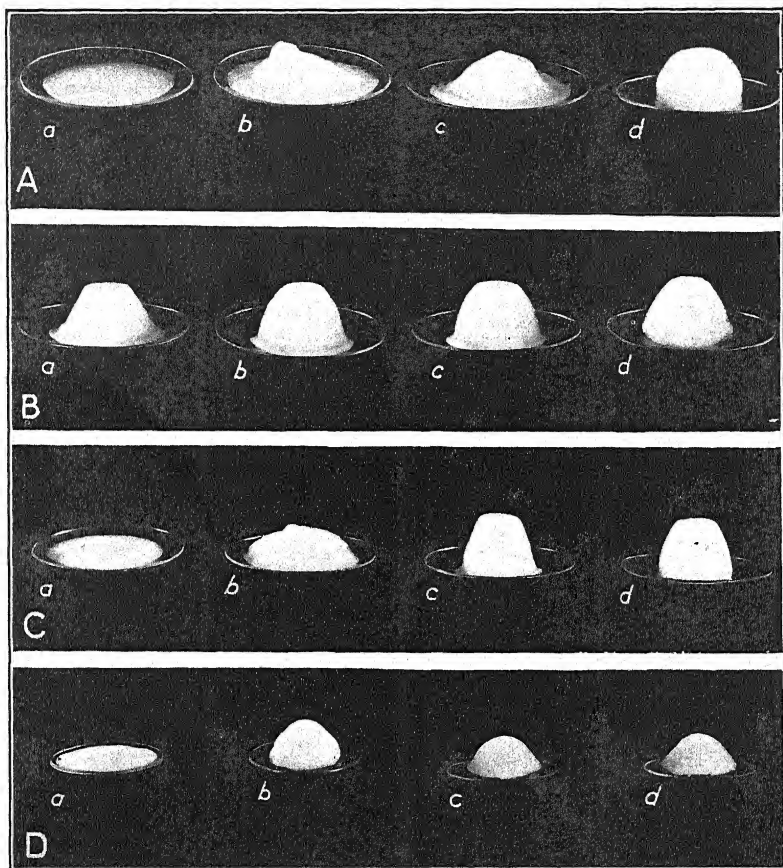


FIGURE 1.—Gels formed by hard-wheat starch: A, cold 5 per cent suspensions heated slowly from room temperature, in electric bath, *a* to 83° C., *b* to 90°, *c* to 90°, and held there 20 minutes, and *d* to 96°; B, hot 5 per cent suspensions each heated differently, *a* in electric bath from room temperature to 90°, *b* in hot electric bath to 90°, *c* in beaker of boiling water to 90°, and *d* to boiling over a flame; C, cold 10 per cent suspensions heated slowly from room temperature, in electric bath, *a* to 70°, *b* to 75°, *c* to 80°, and *d* to 95°; D, cold 50 per cent suspensions heated slowly from room temperature, in electric bath, *a* to 80°, *b* to 55°, *c* to 90°, and *d* to 95°

and prevented movement of the suspension during photographic exposure. Specimens showed no change in many hours when so sealed.

A field, representative of the whole slide, was photographed with a camera attached directly to a polarizing biological microscope carrying a 12× ocular and 30× objective. The field was photographed with ordinary light and a No. B 58 Wratten filter and with an exposure

of three minutes on a panchromatic film. Such fields as showed a considerable number of birefringent granules were also photographed with polarized light, in which case 30 minutes exposure of a nitrate film was required.

### DISCUSSION OF RESULTS

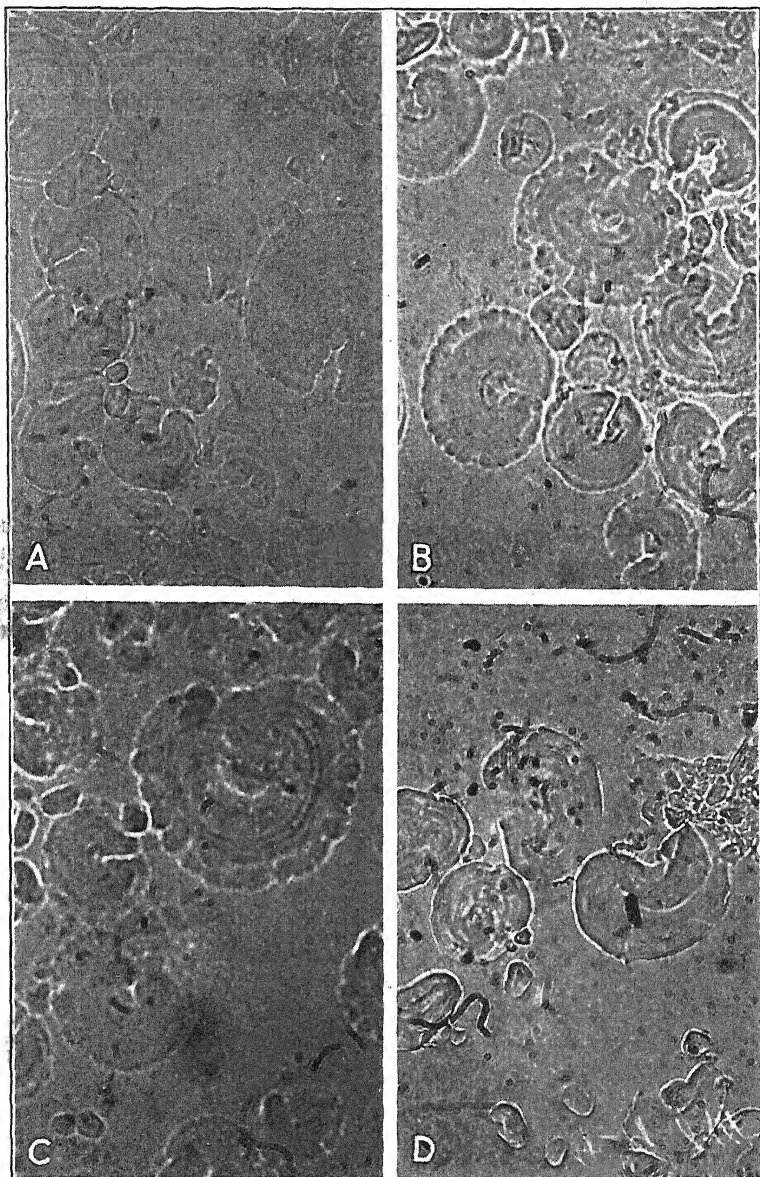
Photomicrographs showing the changes which took place as 5 per cent starch suspensions were heated from room temperature to 96° C. have been made at temperature intervals of 5° for both hard and soft wheat starches, though only a few are reproduced here. These microscopic changes were examined in conjunction with the gel-forming powers of the heated suspension. The ability to form gels was recorded photographically for suspensions heated to temperatures corresponding to those of the suspensions used in the photomicrographs. So little difference was found between hard and soft wheat starches that detailed results for each are not warranted. Hard-wheat starch is shown in the accompanying figures.

### CHARACTERISTICS OF GELS

In the photographs of the gels the outline indicates whether the mass was a firm gel or but a partly formed one. In some of the 5 per cent ones the outline was clear-cut (fig. 1, B, c), but the gel was too weak to resist the pressure of the flat side of a spatula. One as weak as this invariably showed some leaking of water, however, as soon as it was turned out of the mold, and evidence of syneresis was plainly visible in the photograph. The firmest gels obtained rebounded after pressure and showed no syneresis after standing for three to four hours on the watch glass. The weak gels were of granular texture which made the oozing of water easily possible. The firm gels were as homogeneous, so far as the naked eye could detect, as gelatin or pectin ones. This difference in grain unfortunately does not show in the photograph nor does the difference in degree of whiteness or opalescence.

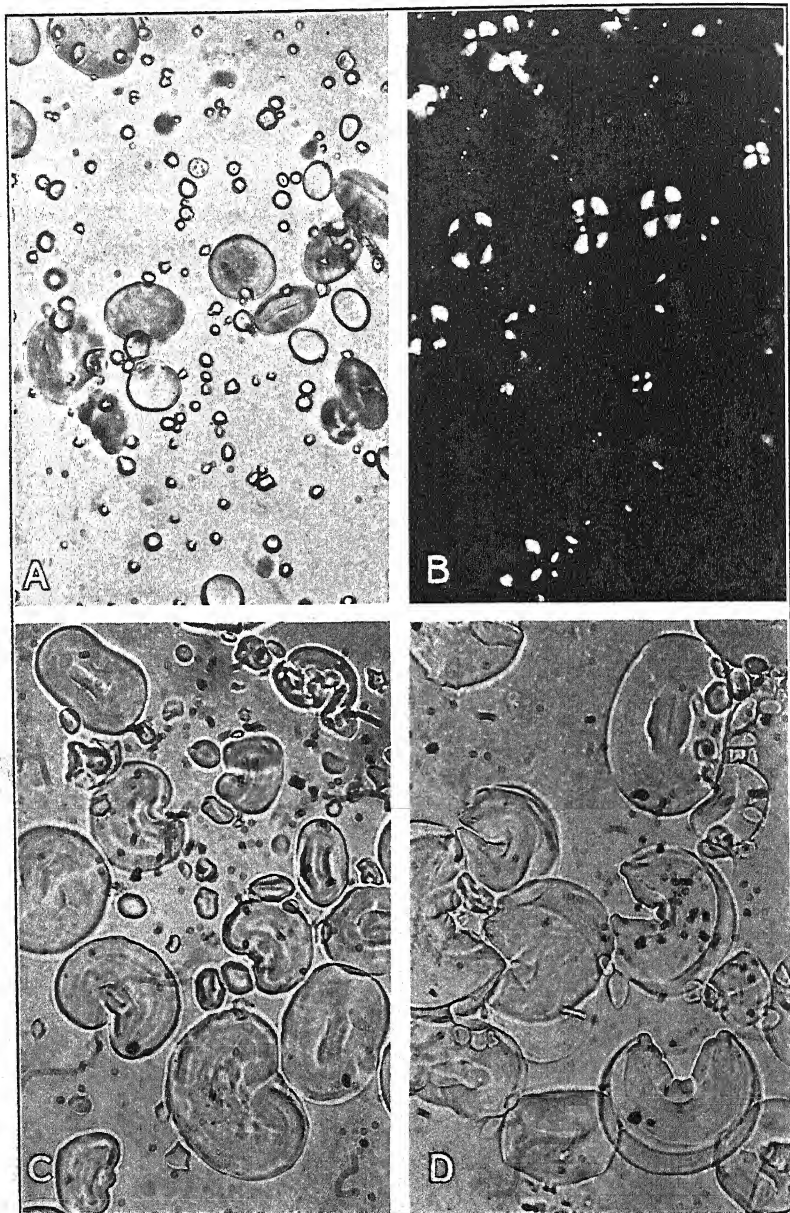
When a starch suspension of 5 per cent concentration was heated to a temperature between 95° and 100° C., a firm gel structure resulted no matter what the rate of heating was. A suspension heated slowly in the electric water bath from a cold state to 96° (fig. 1, A, d,) was less tough, however, than one heated quickly to boiling over a flame. (Pl. 1, B, d.) The temperature of 90° C. seemed to be a critical one for gelation; a gel was never obtained below this temperature (fig. 1, A, a), and gels sometimes failed to form even at 90° if conditions were not properly chosen; also all the gels formed at this temperature showed great weakness and syneresis. Hot suspensions brought rapidly to 90° gave the firmest gels obtainable at this temperature (fig. 1, B, a, b, c); slow heating gave only a suggestion of a gel (fig. 1, A, b), and not much improvement was made even when the heating at 90° was prolonged for 20 minutes. (Fig. 1, A, c.) There was a gradual improvement in gel form as temperatures were increased from 90° to 95°, though no photographs for intervening temperatures are given.

Suspensions containing 10 and 50 per cent starch (fig. 1, C and D) gelled readily at temperatures below those of the 5 per cent one, and their products were of course much tougher. At 80° C. the 10



A, Raw, 5 percent suspension in ordinary light. B, raw, 5 percent suspension in polarized light. C, 5 percent suspension heated to a temperature of 65° C. D, 5 percent suspension heated to a temperature of 75°.  $\times 360$ .





A, 5 percent suspension heated to a temperature of 90° C. B, 5 percent suspension heated to a temperature of 93°. C, 5 percent suspension heated to a temperature of 96°. D, 50 percent suspension heated to a temperature of 95°.  $\times 360$ .



per cent concentration was shapely, and at 53° to 55° the 50 per cent one was. Each was white and raw in appearance at these temperatures, but they became opalescent after the temperature had risen to 95°. No syneresis was seen in either. The soft-wheat starch in the 50 per cent concentration did not become moldable until 60°.

#### MICROSCOPIC CHANGES

Photomicrographs of unheated granules, mounted in water as were the gelatinized ones, are shown with ordinary and polarized light in Plate 1, A and B. Occasional bacterial forms should be overlooked in these water mounted specimens. The grains remained unchanged up through a temperature of 50° C., but at 55° a slight swelling and a disappearance of the dark cross which marks birefringence had become evident in the largest granules. These changes were gradual up to the point of disappearance of birefringence which was 60° for hard, and 65° for soft wheat starch. Also at 65° (pl. 1, C), the granules had become very swollen, and a few were assuming a characteristic kidney shape which seemed to be due to a swelling of the granule around a constriction reaching to the center. This shape became more pronounced as the temperature rose, and at 93° the outer edges became beaded with what appeared to be perforations. (Pl. 1, D and 2, B.) This effect could be seen either with or without a cover glass, so it is unlikely that its cause was outward pressure upon the swollen particle. The general appearance was very similar in the two starches. In 50 per cent concentration birefringence disappeared from hard-wheat starch at 75° but did not disappear from soft-wheat starch until a temperature of 85° was reached.

The appearance assumed by granules in the gels at 90° C., which in 5 per cent strength were always weak, is shown in Plate 2, A. Water was apparently not held tightly by them so long as they retained this appearance, as evidenced by the fact that syneresis was prominent. Firm, syneresis-free gels are shown in Plate 2, C. Their grains were so swollen that they were blurred and were brought into focus with difficulty. This was not true of the 50 per cent concentration heated to 95°. Plate 2, D shows the grains were swollen but much less so, and birefringence had left the smallest ones at only 85°.

The tenacity with which the swollen granules held their water seemed to depend upon the amount of it at their command, upon the temperature to which they were finally heated, and upon the rapidity with which this temperature was reached. Where little water was available for each grain, as in the 50 per cent pastes, it was all held tightly so that no syneresis could be seen. This was true even though the microscope showed not much more swelling of the grains than had occurred in 5 per cent suspensions heated to only 65° or 70° C. Photomicrographs are not shown of the almost shapeless and indistinguishable masses characteristic of the 5 per cent pastes heated rapidly to boiling. This method, it has already been said, gave the firmest gel, most nearly free from syneresis. Why the granules absorbed the water more abundantly and more permanently at the faster rate of heating and at the higher temperature will require more work to explain.

Translucency was not a requisite of a moldable paste in the two higher concentrations studied. In the 5 per cent concentration a good

gel did not form until a temperature several degrees higher than that of the region of beginning translucency had been reached. The moldability of the 10 and 50 per cent pastes at low temperatures can probably be accounted for by the fact that many grains swelled a little as contrasted with the 5 per cent ones where fewer grains swelled much.

These results showed that the temperature at which birefringence disappeared depended on the quantity of water available to the granules. Anisotropy was gone long before the 5 per cent suspension would gel and persisted long after the 50 per cent one was a solid mass.

There was nothing in any of these observations on the physical properties of hard and soft wheat starches that would lead one to expect differences in cooking results to be due to them.

### SUMMARY

Some relationships of concentration, temperature of heating, microscopic changes, and ability to form a moldable gel have been recorded in the form of photomicrographs of granules and photographs of gels given by starches of soft and hard wheats.

Photomicrographs of starch, raw and heated in 5 per cent suspension to 55°, 75°, 90°, 93°, and 96° C. are reproduced showing characteristics which have not heretofore been described in the literature.

A temperature of 90° C. seemed to be a critical one for gelation of a 5 per cent suspension. Rapid heating to this temperature gave a better-formed gel than did slow heating. Temperatures of 95° or above gave the firmest gels obtained with this concentration. Photographs show by outline the firmness of gels and suggest the comparative amounts of syneresis.

In 50 per cent concentrations, heating to 95° C. caused no greater swell than did a temperature of 65° or 70° in a 5 per cent suspension. The 50 per cent concentration became moldable at 53° to 55° even though birefringence persisted up to 75°. Anisotropy disappeared at 60° in the 5 per cent suspension.

The tenacity with which the swollen granules held their water seemed to depend on the amount of it at their command, on the temperature to which they were finally heated, and on the rapidity with which this temperature was reached. Ten and fifty per cent suspensions became moldable at temperatures below the region of appearance of translucency. This was not true of the 5 per cent suspension. Many grains swelling a little apparently accomplished somewhat the same solid effect in the high concentrations as did fewer grains swelling much in the lower one.

No significant difference was found in the gels of hard and soft wheat starches.

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# MILK-ENERGY FORMULAS FOR VARIOUS BREEDS OF CATTLE<sup>1</sup>

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## INTRODUCTION

The calorific value of milk is important as a measure of the human food value of the milk, the amount of feed required by the cow for lactation, and as a measure of lactation for the study of inheritance of milking ability.

Direct determination of the calorific value of milk has not been carried out very extensively. Overman and Sanmann (8, 9)<sup>2</sup> have reported analyses, including energy, of 212 samples without classifying them as to the breed of cow. Similar data have been reported by Möllgaard (7) on 60 samples from Red Danish cows and 11 samples from Jersey cows; by Kahlenberg and Voris (6) on 134 samples from Holstein cows; and by Savini and Gargia (11) on about 100 samples of market milk collected in the retail trade. All these investigators have pointed out the possibility of estimating the energy value of milk from its fat percentage. Overman and Sanmann found the correlation between fat percentage and calories per kilogram of milk to be  $r = 0.9814 \pm 0.0017$ .<sup>3</sup> That is to say, the energy content of the whole milk produced by the cow may be estimated with a high degree of accuracy from its weight and fat percentage. The equations are given in Table 3.

Kahlenberg and Voris derived the formula,  $E = 183f - 56.73t + 556.32$ , where  $E$  is calories per kilogram of milk,  $f$  is the percentage of fat and  $t$  is the percentage of solids not fat. According to this equation, at a given fat percentage increasing the solids not fat decreases the energy value, which does not seem reasonable. Also, inspection of their data suggests that the coefficient of  $t$  should be positive. For example, there are four observations at  $f = 3.76$  and within these four we find:

Percentage of solids not fat.....	7.68	8.69	8.92	8.95
Calories per kilogram of milk, determined.....	667	705	710	720

In this particular instance, at least, energy tends to increase with the solids not fat.

The writers have taken the trouble to recompute the formula from the published analyses, without coding, by use of the method described by Brandt (2). The results are  $R_{E,f} = 0.9930$ , and  $E = 93.68f + 44.55t - 34.09$ . In this equation the coefficients of  $f$  and  $t$  are both positive and substantially in the ratio of the average energy values of fat and solids not fat, while the constant factor is very small as compared with

<sup>1</sup> Received for publication Jan. 4, 1933; issued July, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 1120.

<sup>3</sup> This value was computed by coding. Use of their published data without coding gives  $r = 0.9874$ , a substantially higher value. When dealing with such highly correlated variables it is desirable to use small class intervals.

that of Kahlenberg and Voris. By using the above example again, results from the two formulas in comparison with the determined energy values are:

Kahlenberg and Voris formula.....	809	751	738	737
Determined.....	667	705	710	720
Present formula.....	660	705	716	717

Clearly the present formula agrees with the observations much better than does the formula of Kahlenberg and Voris.

While the data of Overman and Sanmann were not classified as to breed directly, the fat-percentage classification itself would automatically make some breed separation. The plotted values showing the regression of energy on fat percentage seem to be entirely linear, with no evidence of any breed influence. On the other hand, the equations of Möllgaard and Kahlenberg and Voris suggest that there may be breed differences of some significance with respect to the relation between energy and fat percentage. What is needed is an extension of these direct determinations to a larger number of samples and especially to a larger population of cows in each of the several breeds. However, it seems worth while as a preliminary to a study of breed differences to see what information may be gained from the analyses of milk of the different breeds. Such information may have value also in itself.

In the present paper certain analyses from this laboratory are presented, and a comparison is made between the different breeds with respect to milk energy. The analyses used are the 212 reported by Overman and Sanmann (8, 9) which include direct energy determinations, and another set of 1,999 by Overman, Sanmann, and Wright (10), classified by the breed of cow, and similar to the 212 except that they do not include direct energy determinations. The energy of the milk components was derived from the 212 analyses, and these component values were then applied to the 1,999 analyses in order to derive the milk energy.

#### ENERGY OF MILK COMPONENTS

The energy of milk obviously lies in its several components. If both the milk energy and the amounts of the several milk components are known for a series of observations, it should be possible to deduce the energy factor for each component. It should then be permissible to apply these factors to other analyses in which energy was not determined and thereby estimate the milk energy.

It may be assumed that all of the 212 analyses including energy are equally reliable and that such discrepancies as may exist among them with respect to the present point of view are due to errors or differences of a random nature; then it may be assumed that the most probable energy values of the several components may be determined by applying the principle of least squares to the whole series of observed values. For illustration, if the energy of milk resided entirely in the fat, protein, and lactose and absolutely accurate determinations of these items in three divergent samples of milk were available, as well as the accompanying milk energy, then it would be merely necessary to set up the results as three equations and solve for the energy value of fat, protein, and lactose. It is clearly not justifiable to pick 3 out of the 212 to be used in preference to any other 3. The theoretic-

cally correct procedure is to form a set of normal equations from the 212 observations and thus arrive at the proper values from all the observations. This procedure has been followed, by using the method described by Brunt (3) and assuming that the energy resides in (1) fat, protein, and lactose; (2) fat, protein, lactose, and ash; and (3) fat, protein, lactose, ash, and water. The results are presented in Table 1.

TABLE 1.—*Energy value of milk components in calories per gram*

Component	Values based on energy residing in—			Standard values reported by Abderhalden
	Fat, protein, lactose, ash, and water	Fat, protein, lactose, and ash	Fat, protein, and lactose	
Fat.....	9.312	9.434	9.253±0.065	9.23
Protein.....	5.358	5.161	5.853±.127	5.71
Lactose.....	3.987	3.480	3.693±.059	3.95
Ash.....	4.980±0.199	4.323±0.173		
Water.....	-.0350±.0059			

Table 1 shows that the values found for fat, protein, and lactose fluctuate to some extent according to the components considered in calculating the values. This suggests that the method is not adapted to the exact estimation of the energy of milk components. The standard values for fat, protein, and lactose directly determined, as given by Abderhalden (1), are given in the last column. The present indirectly determined values, based on fat, protein, and lactose, agree very closely with the standard values in the case of fat but poorly in the case of lactose. Lactose was determined by difference, and the poor agreement perhaps means that the errors of determination thus tend to concentrate in this portion. The good agreement of the fat values, on the other hand, may reflect a superior accuracy in the fat determination, along with the high proportion of the total milk energy represented by the fat.

It might be concluded in advance that the water has no energy value and should be left out of consideration. Statistically the value found is significant although absolutely so small that the result may be regarded as a favorable comment on the consistency of the analyses. Likewise, the ash might have been ruled out in advance as having no appreciable energy value. On analysis, the value found, while appreciable per gram, becomes of little consequence when considered in connection with the small quantity of ash present in the milk.

The question now arises as to which set of values in Table 1 may best be used in estimating milk energy from the chemical analyses. A prior one might conclude that water should certainly be excluded, and probably ash also, fat, protein, and lactose being chosen as the most rational set. We are concerned, however, in making the most accurate estimate we can of the milk energy from the milk analyses. It is not at all inconceivable that, while there is no "negative energy" to be attributed to the water of the milk as analyzed, there may nevertheless be some peculiarity associated with high-water milk which tends to make the energy determination run slightly lower. Similar reasoning may be applied to the ash. Since the analyses, without energy, which it is proposed to use, are entirely comparable with those

involved in Table 1, it seems clear that statistically the best energy estimate will be obtained by the use of all five components—that is,  $E = 93.12f + 53.58p + 39.87l + 49.80a - 0.356w$ , where  $E$  is calories per kilogram of milk and  $f$ ,  $p$ ,  $l$ ,  $a$ , and  $w$  are the percentages of fat, protein, lactose, ash, and water, respectively.

### BREED FORMULAS

The above formula has been applied to the milk analyses, classified by breed, as published by Overman, Sanmann, and Wright (10). The analyses for each breed were grouped by fat percentage into classes, 2.60–2.79, 2.80–2.99, 3.00–3.19, etc., and the average of the analyses of each group determined. The formula was then applied to these averages to derive the energy values. The results are given in Table 2.

TABLE 2.—Computed energy values and protein-energy ratios of milk from cows of various breeds and from crossbred cows, grouped by fat-percentage classes

#### AYRSHIRE COWS

Fat-percentage class	Records	Fat	Protein	Lactose	Ash	Water	Energy per kilogram of milk	Protein per calorie
	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Calories	Milli-grams
2.80 to 2.99.....	2	2.920	3.020	4.625	0.658	88.78	619.3	49.0
3.00 to 3.19.....	3	3.113	3.170	4.607	.694	88.42	646.5	49.0
3.20 to 3.39.....	12	3.327	3.321	4.553	.679	88.12	671.7	49.5
3.40 to 3.59.....	12	3.506	3.314	4.689	.655	87.84	692.3	47.9
3.60 to 3.79.....	27	3.706	3.325	4.673	.659	87.64	711.2	46.8
3.80 to 3.99.....	24	3.900	3.519	4.551	.688	87.34	736.3	47.8
4.00 to 4.19.....	34	4.085	3.635	4.772	.684	86.82	768.6	47.3
4.20 to 4.39.....	34	4.292	3.576	4.609	.686	86.85	778.3	45.9
4.40 to 4.59.....	20	4.498	3.720	4.821	.691	86.27	814.1	45.7
4.60 to 4.79.....	19	4.691	3.802	4.708	.695	86.10	832.2	45.7
4.80 to 4.99.....	7	4.863	3.794	4.683	.702	85.96	847.2	44.8
5.00 to 5.19.....	5	5.112	3.888	4.948	.703	85.35	886.3	43.9
5.20 to 5.39.....	5	5.314	3.976	5.026	.707	84.98	913.2	43.5
5.40 to 5.59.....	3	5.490	4.140	5.007	.753	84.61	940.1	44.0
5.60 to 5.79.....	1	5.66	4.38	4.80	.730	84.43	959.4	45.7

#### GUERNSEY COWS

3.60 to 3.79.....	3	3.720	3.727	4.963	0.702	86.89	748.0	40.8
3.80 to 3.99.....	7	3.914	3.360	4.684	.701	87.34	735.1	45.7
4.00 to 4.19.....	10	4.078	3.561	4.995	.728	86.64	775.1	45.9
4.20 to 4.39.....	15	4.297	3.661	4.853	.735	86.45	795.6	46.0
4.40 to 4.59.....	29	4.499	3.663	4.965	.717	86.15	818.2	44.8
4.60 to 4.79.....	38	4.689	3.757	4.958	.716	85.88	840.7	44.7
4.80 to 4.99.....	36	4.894	3.907	4.978	.733	85.49	869.6	44.9
5.00 to 5.19.....	39	5.149	3.992	4.958	.737	85.21	897.4	44.5
5.20 to 5.39.....	35	5.274	4.015	4.924	.745	85.05	909.4	44.1
5.40 to 5.59.....	24	5.485	4.199	4.927	.761	84.63	940.0	44.7
5.60 to 5.79.....	22	5.693	4.165	4.921	.757	84.46	957.1	43.5
5.80 to 5.99.....	19	5.897	4.253	4.922	.753	84.17	980.8	43.4
6.00 to 6.19.....	12	6.068	4.612	4.711	.770	83.78	1,008.5	45.6
6.20 to 6.39.....	11	6.285	4.555	4.761	.773	83.62	1,027.9	44.3
6.40 to 6.59.....	9	6.472	4.611	4.754	.804	83.36	1,049.6	43.9
6.60 to 6.79.....	5	6.662	4.770	4.740	.788	83.04	1,074.6	44.4
6.80 to 6.99.....	3	6.877	4.847	4.743	.829	82.70	1,101.0	44.0
7.00 to 7.19.....	1	7.06	4.98	4.78	.768	82.41	1,123.7	41.1
7.20 to 7.39.....	1	7.37	4.70	4.88	.816	82.23	1,144.0	41.1
7.60 to 7.79.....	2	7.650	4.790	4.605	.812	82.14	1,163.8	41.2



TABLE 2.—*Computed energy values and protein-energy ratios of milk from cows of various breeds and from crossbred cows, grouped by fat-percentage classes—Continued*

HOLSTEIN COWS								
Fat-percentage class	Records	Fat	Protein	Lactose	Ash	Water	Energy per kilogram of milk	Protein per calorie
	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Calories	Milli-grams
2.60 to 2.79.....	8	2.709	3.055	5.000	0.656	88.58	616.4	49.6
2.80 to 2.99.....	25	2.890	3.012	4.928	.666	88.51	628.6	47.9
3.00 to 3.19.....	40	3.104	3.169	4.846	.672	88.21	654.1	48.4
3.20 to 3.39.....	47	3.289	3.276	4.884	.671	87.88	678.6	48.3
3.40 to 3.59.....	42	3.488	3.388	4.853	.688	87.58	702.9	48.2
3.60 to 3.79.....	35	3.674	3.361	4.866	.672	87.43	718.5	46.8
3.80 to 3.99.....	29	3.874	3.502	4.863	.681	87.08	745.2	47.0
4.00 to 4.19.....	11	4.078	3.734	4.858	.694	86.64	777.2	48.0
4.20 to 4.39.....	13	4.313	3.967	4.722	.697	86.30	806.4	49.2
4.40 to 4.59.....	5	4.510	4.240	4.998	.736	85.51	852.6	49.7
4.60 to 4.79.....	4	4.700	4.075	4.757	.692	85.77	849.6	48.0
5.03 to 5.19.....	3	5.156	4.586	4.730	.735	84.79	920.8	49.8
5.20 to 5.39.....	3	5.306	5.010	4.833	.804	84.05	965.3	51.9
5.60 to 5.79.....	2	5.680	5.420	4.490	.812	83.60	1,009.0	53.7
6.00 to 6.19.....	1	6.00	4.88	4.66	.794	83.67	1,015.7	48.0

JERSEY COWS								
3.20 to 3.39.....	1	3.280	3.22	3.67	0.789	89.04	631.9	51.0
3.60 to 3.79.....	5	3.680	3.896	3.942	.729	87.75	713.7	54.6
3.80 to 3.99.....	8	3.910	3.623	4.860	.683	86.92	755.1	48.0
4.00 to 4.19.....	5	4.074	3.302	4.866	.660	87.10	752.2	43.9
4.20 to 4.39.....	11	4.292	3.695	5.126	.697	86.19	806.1	45.8
4.40 to 4.59.....	18	4.493	3.648	5.058	.691	86.11	819.3	44.5
4.60 to 4.79.....	16	4.608	3.556	5.059	.681	86.03	830.2	42.8
4.80 to 4.99.....	20	4.881	3.715	5.058	.693	85.65	859.3	43.2
5.00 to 5.19.....	19	5.091	3.896	4.919	.694	85.40	883.1	44.1
5.20 to 5.39.....	26	5.295	3.859	5.026	.698	85.12	904.7	42.7
5.40 to 5.59.....	16	5.479	3.891	4.986	.693	84.95	921.7	42.2
5.60 to 5.79.....	12	5.707	4.171	4.963	.743	84.42	959.7	43.5
6.80 to 5.99.....	12	5.892	4.288	4.929	.732	84.16	981.4	43.7
6.00 to 6.19.....	8	6.081	4.264	4.764	.726	84.17	990.9	43.0
6.20 to 6.39.....	5	6.230	4.010	4.982	.713	84.06	999.2	40.1
6.40 to 6.59.....	4	6.445	4.068	4.788	.727	83.97	1,015.3	40.1
7.60 to 6.79.....	5	6.666	4.008	5.042	.709	83.58	1,042.1	38.5
7.80 to 6.99.....	1	6.80	4.44	4.96	.698	83.10	1,074.0	41.3
8.20 to 7.39.....	1	7.34	4.91	4.06	.650	83.04	1,111.3	44.2
5.40 to 7.59.....	1	7.59	3.23	5.15	.706	83.32	1,090.7	29.6
8.20 to 8.39.....	2	8.365	4.925	3.605	.772	82.33	1,195.7	41.2

CROSSBRED COWS								
2.60 to 2.79.....	1	2.72	3.27	3.91	0.682	89.42	586.5	55.8
2.80 to 2.99.....	4	2.933	2.915	5.113	.682	88.36	635.7	45.9
3.00 to 3.19.....	19	3.085	3.103	5.093	.689	88.03	659.6	47.0
3.20 to 3.39.....	19	3.295	3.203	5.048	.685	87.77	682.6	46.9
3.40 to 3.59.....	61	3.497	3.466	4.987	.697	87.35	713.8	48.6
3.60 to 3.79.....	89	3.700	3.514	4.929	.711	87.15	733.7	47.9
3.80 to 3.99.....	111	3.895	3.560	4.917	.716	86.91	754.2	47.2
4.00 to 4.19.....	125	4.099	3.622	4.878	.723	86.68	775.4	46.7
4.20 to 4.39.....	110	4.293	3.790	4.894	.723	86.30	803.2	47.2
4.40 to 4.59.....	130	4.489	3.864	4.820	.723	86.10	822.6	47.0
4.60 to 4.79.....	93	4.690	3.918	4.833	.739	85.82	845.6	46.3
4.80 to 4.99.....	70	4.895	4.046	4.820	.737	85.50	871.0	46.5
5.00 to 5.19.....	60	5.090	4.149	4.834	.743	85.18	895.7	46.3
5.20 to 5.39.....	42	5.278	4.275	4.665	.739	85.04	913.1	46.8
5.40 to 5.59.....	27	5.477	4.480	4.818	.761	84.46	950.0	47.2
5.60 to 5.79.....	17	5.679	4.422	4.558	.769	84.57	955.7	46.3
5.80 to 5.99.....	11	5.874	4.735	4.383	.756	84.25	983.1	48.2
6.00 to 6.19.....	2	6.120	4.835	4.640	.776	83.63	1,022.8	47.3
6.20 to 6.39.....	4	6.290	4.655	4.658	.769	83.63	1,029.4	45.2
6.40 to 6.59.....	6	6.472	4.653	4.747	.798	83.33	1,051.3	44.3
7.40 to 7.59.....	1	7.50	5.54	4.04	.814	82.11	1,167.6	47.4

The equation  $E = a + bf$  has been fitted to the average fat-percentage and energy values of each of these tables by the method of least squares, and weighting by the number of records at each fat-percentage class. The equations are given in Table 3, together with the directly determined equations of the investigators referred to at the outset.

TABLE 3.—Milk-energy formulas for various breeds of cows

Breed	Formula <sup>a</sup>	Authority
Not classified.....	$E = 115.33 (2.51 + f)$ .....	Overman and Sanmann.
Red Danish.....	$E = 115 (2.44 + f)$ .....	Møllgaard.
Jersey.....	$E = 101 (3.59 + f)$ .....	Do.
Holstein.....	$E = 128.55 (1.89 + f)$ .....	Kahlenberg and Voris.
Jersey.....	$E = 106.98 (3.15 + f)$ .....	Present paper.
Holstein.....	$E = 128.19 (1.99 + f)$ .....	Do.
Guernsey.....	$E = 116.80 (2.52 + f)$ .....	Do.
Ayrshire.....	$E = 121.00 (2.20 + f)$ .....	Do.
Crossbred.....	$E = 116.11 (2.61 + f)$ .....	Do.

<sup>a</sup>  $E$ =calories per kilogram milk,  $f$ =percentage fat content of milk.

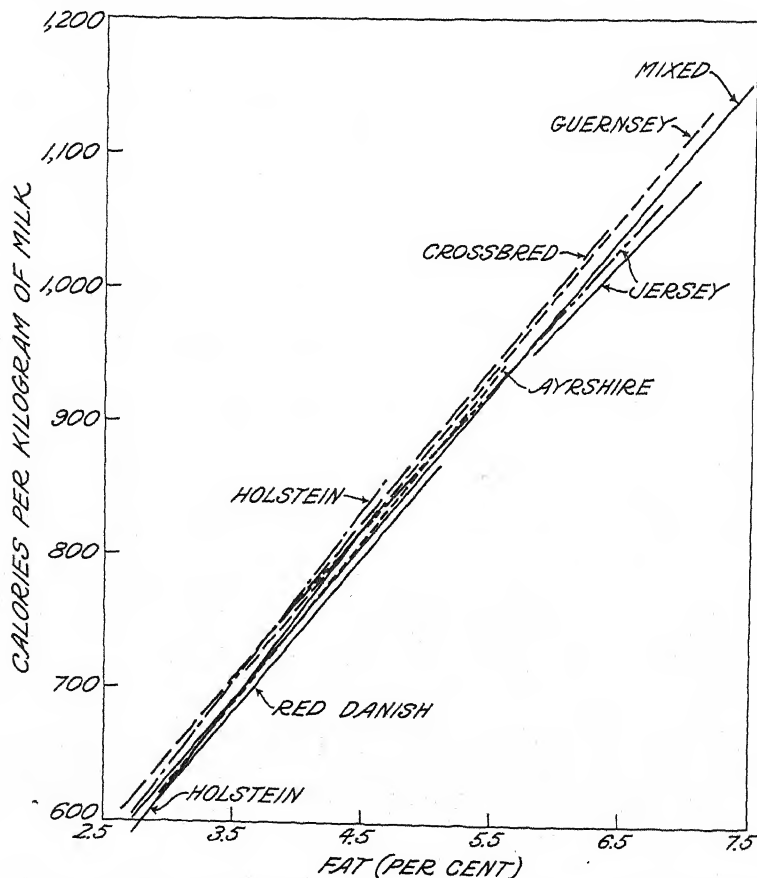


FIGURE 1.—Fitted curves showing relation of milk energy to fat percentage. The broken lines represent data of this paper. Equations in Table 3

A study of this table suggests that there is undoubtedly some difference in the slopes of the breed curves. The Holstein breed has the steepest curve and the Jersey breed the flattest. The slope of the present indirectly determined curve for the Holstein breed agrees very closely with the directly determined data of Kahlenberg and Voris. The similar comparison with Möllgaard's Jersey data shows somewhat less exact agreement, but the results are in accord in indicating a difference between the two breeds.

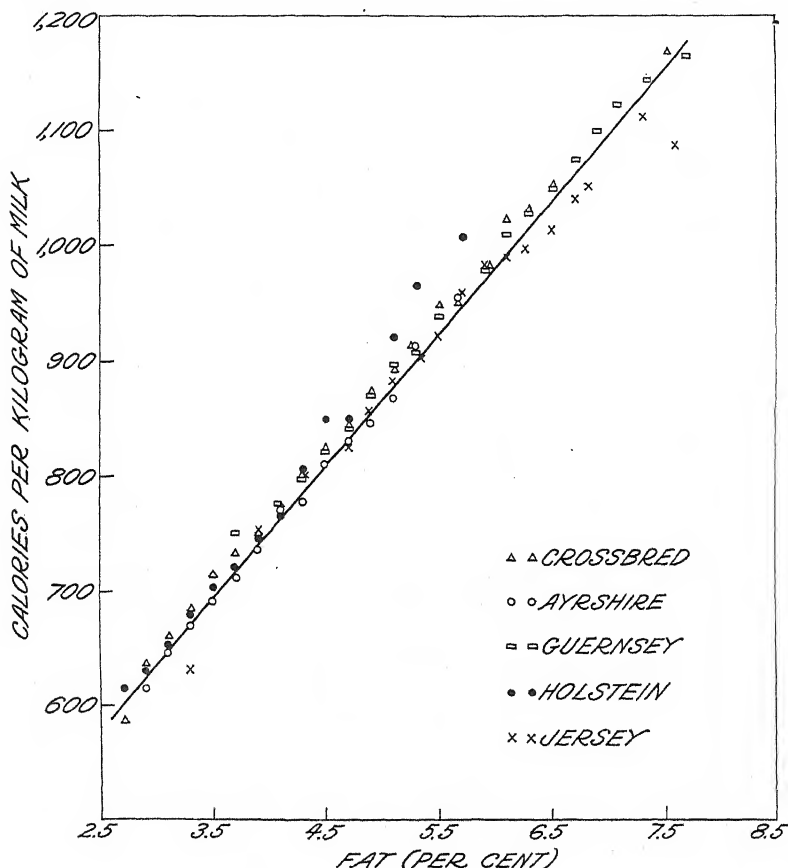


FIGURE 2.—Observations of Table 2 showing relation of milk energy to fat percentage. The line represents Overman and Sanmann's equation

The curves of the equations of Table 3 are plotted in Figure 1, which gives a better general view of the variation between breeds than do the equations. The curves are plotted to cover the range of fat percentage found in the observed data on which they are based. Considered within these limits the curves all lie in a comparatively narrow band with slope practically the same as that of Overman and Sanmann's mixed data. While the difference in breeds may be of real significance, there is no great error involved, within

the usual fat-percentage range of any breed, by using a single equation to describe the fat-energy relation for all breeds.

The computed energy values of Table 2 are plotted against fat percentage in Figure 2. The curve of Overman and Sanmann's equation is also given as a guide. It is apparent that a majority of the observations lie above this line—that is, the equation gives values slightly too low to fit the observed values. This figure brings out a difference between high-testing Holstein and Jersey samples. High-testing Holstein samples tend to show an energy content above that expected, while the high-testing Jersey samples tend to show an energy content below that expected. In general, Figure 2 shows that breed differences are slight for samples testing within the usual fat-percentage range of the breed.

#### PROTEIN-ENERGY RATIO

Fredericksen (4) has pointed out that the ratio of protein to energy is nearly the same regardless of the natural fat-content percentage of the milk. This observation is of prime importance from the point of view of the whole-milk trade and from the point of view of dairy feeding standards. The protein-energy ratio has been computed for each fat-percentage class and is given in the last column of Table 2. The ratios for each breed have been connected with the fat percentage by means of the equations given in the following tabulation. The equations were fitted by the method of least squares weighting by the frequencies.

Breed	Milligrams of protein per calorie	Breed	Milligrams of protein per calorie
Holstein.....	46.49+0.46 <i>f</i>	Ayrshire.....	56.29-2.32 <i>f</i>
Crossbred.....	49.68- .61 <i>f</i>	Jersey.....	55.59-2.32 <i>f</i>
Guernsey.....	48.89- .83 <i>f</i>	Jersey <sup>4</sup> .....	47.93- .87 <i>f</i>

The observed ratios and fitted curves are shown graphically in Figures 3 and 4, and all the fitted curves are brought together in Figure 5. There seem to be marked differences between the breeds in the change of the protein-energy ratio with change in fat percentage. The Holstein breed shows a general tendency for the ratio to increase with increase of fat percentage, while the Ayrshire breed shows a marked decrease in the proportion of protein to energy as the fat percentage increases. It seems safe to say that the Ayrshire and Holstein breeds are significantly different in the protein-energy ratio of their milk. It may be noted that the Holstein observed values (fig. 3) are somewhat irregular, while the Ayrshire values (fig. 4) conform very closely to the fitted curve throughout the range.

According to the fitted curves as shown in Figure 5, the Ayrshire and Jersey breeds resemble each other and stand more or less apart from the other breeds. Reference to Figure 4 suggests, however, that the Jersey curve may be influenced to a considerable degree by the observations below  $f=4.10$ , and above  $f=6.10$ . If these observations, representing 33 analyses or 16.5 per cent of the total, are excluded a much flatter curve is obtained, as shown by the broken line in Figures 4 and 5. This limited Jersey curve resembles the Guernsey curve. The data therefore leave one in doubt as to the genuineness of the resemblance of the Jersey curve to the Ayrshire

<sup>4</sup> For limits of  $f=4$  to  $f=6.19$ .

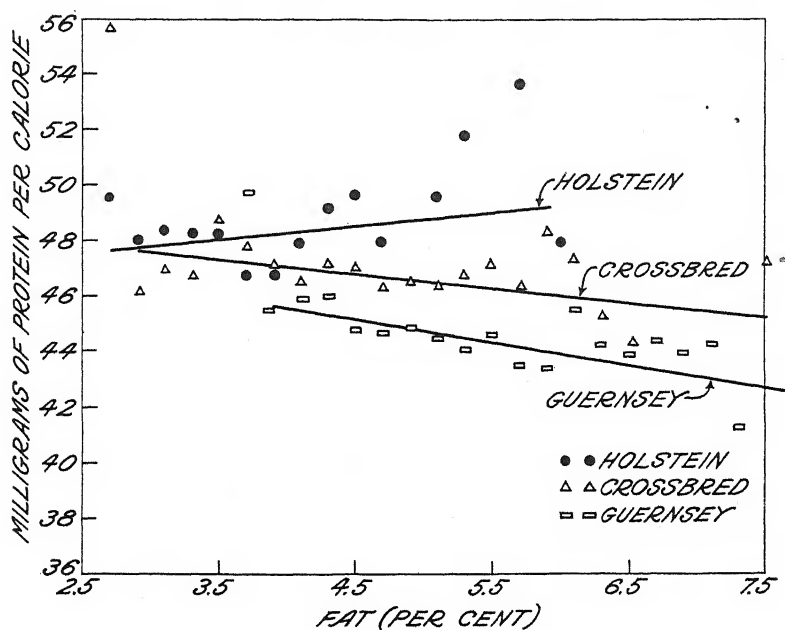


FIGURE 3.—Relation between the protein-energy ratio and the fat percentage of the milk from Guernsey and Holstein breeds and crosses of these two breeds. The lines represent the fitted equations

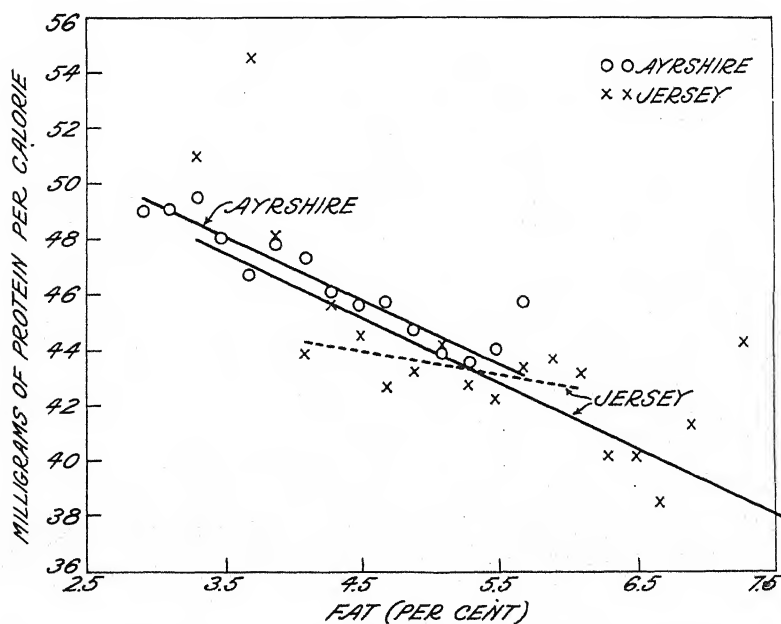


FIGURE 4.—Relation between the protein-energy ratio and the fat percentage of the milk from Ayrshire and Jersey breeds. The solid lines represent the fitted equations; the broken line applies to the Jersey data over the limited fat-percentage range

curve. It would be more natural on the basis of ancestral descent to accept the resemblance to the Guernsey curve.

Generally speaking, the present ratios indicate that the amount of protein in whole-milk samples from cows of all breeds lies between 43 and 49 mg of protein per calorie. If we were to deal with the lactation milk yield of individual cows, or mixed herd milk, this range would undoubtedly be considerably reduced. So far as the whole-milk trade is concerned it is apparent that the reduction of normal whole milk to a calorie basis places it on a food basis not only with respect to total nutrients, but also, substantially, with

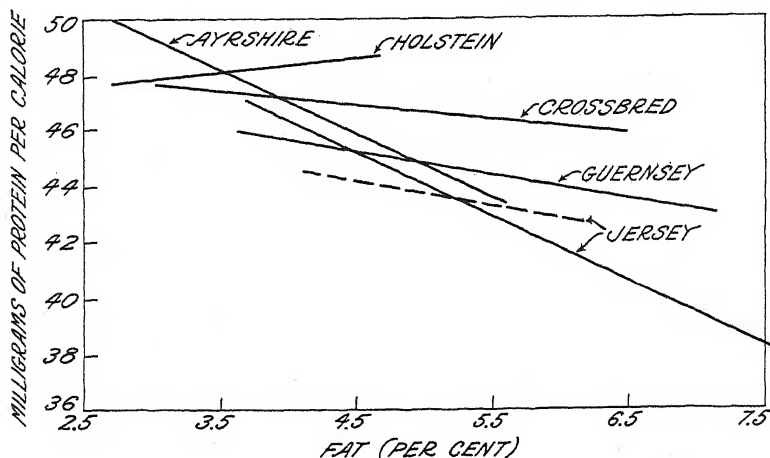


FIGURE 5.—Fitted curves showing the relation between the protein-energy ratio and the fat percentage of milk from different breeds of cattle. The broken line applies to the Jersey data over the limited fat-percentage range. The equations are given in the text

respect to its protein content. So far as feeding standards for milk cows are concerned, it is apparent that on a calorie basis the variation in protein content of the milk is practically negligible.

#### SIMPLIFIED DAIRY FEEDING STANDARD

The feed requirements for milk production are generally given in table form, listing numerous fat-percentage classes of milk. It has been shown (5, fig. 4) that the total nutrients required for lactation are closely proportional to the milk energy (Haecker's law). Since the milk protein is also substantially proportional to milk energy, it follows that the main food requirements, total digestible nutrients, and protein can be expressed as very simple functions of the milk energy.

One way of expressing milk energy is in terms of 4 per cent milk by the formula, 4 per cent milk =  $0.4M + 15F$ , where  $M$  is milk and  $F$  is fat, all in the same unit of weight. On the basis of 750 calories per kilogram of 4 per cent milk this formula is practically the same as that of Overman and Sanmann. Figure 1 shows that this formula is an adequate expression for all the breeds from a practical standpoint. One kilogram of 4 per cent milk, natural or by the  $0.4M + 15F$  formula, contains 750 calories and 37 g of protein, on the basis of 50 mg

of protein per calorie. Allowing a 100 per cent margin for calories and a 50 per cent margin for protein (in line with current feeding standards), the lactation requirement for 1 kg of 4 per cent milk would be, therefore, 1,500 calories of digestible feed energy and 55 g of digestible protein. This principle is embodied in a plan for the teaching and practice of feeding dairy cows which is widely used in the Scandinavian countries at the present time.

So far as dairy cows alone are concerned, it would appear entirely sufficient to determine for the various feeding stuffs simply the content of energy and protein and the digestibility coefficient for these two items. The feeding value might then be expressed in terms of digestible energy and digestible protein, which as above pointed out, bear a definite ratio to the 4 per cent milk requirements. This would reduce the amount of work necessary in feed analyses and digestion trials.

#### INHERITANCE ASPECTS

From an inheritance point of view it is interesting to compare the curves of Figures 1 and 5 relating to cows of the Guernsey and Holstein breeds and the crossbred cows resulting from the crossing of these two. With regard to the relation of milk energy to fat percentage the difference between the parental breed curves is not great, and there is consequently little chance for clear-cut results in the cross. The slope of the crossbred curve is the same as that of the Guernsey breed and somewhat less than that of the Holstein breed.

In the case of the protein-energy ratio, however, there is more difference in the parental breeds, and the crossbred cows take more or less distinctly an intermediate position. (Fig. 3.) It should be mentioned that the Guernsey and Holstein samples are not from the actual parents of the crossbred cows, but only from cows of the same breed as the parents.

#### SUMMARY

Milk-energy formulas for the Ayrshire, Guernsey, Holstein, and Jersey breeds, and Guernsey-Holstein crossbred cows have been derived from analyses available from this laboratory. The analyses (212), including a direct energy determination, were used to determine the energy value of the several milk components. The values were then applied to another, more extensive series of analyses (1,999), and the milk energy was thus indirectly determined. Based on fat, protein, and lactose only, the following values in calories per gram were found: Fat,  $9.253 \pm 0.065$ ; protein,  $5.853 \pm 0.127$ ; lactose,  $3.693 \pm 0.059$ .

The breed formulas for the estimation of milk energy based on the fat percentage showed small but probably significant differences. As compared with the original formula of Overman and Sanmann the breed formulas, considered within the usual fat-percentage limits, all lay within a narrow band on either side. It is concluded that for practical purposes it is permissible to use a single formula for all breeds, which may be expressed in terms of 4 per cent milk as  $4 \text{ per cent milk} = 0.4M + 15F$ , in which  $M$  is the weight of milk and  $F$  is the weight of fat, all in the same unit. One kilogram of 4 per cent milk = 750 calories, or perhaps slightly more.

The change in the protein-energy ratio with change in fat percentage showed more marked breed differences than were found for milk energy. Crossbred cows were intermediate as compared with the parent breeds. In a rough way the protein-energy ratio may be considered constant, lying usually within the limits of 43 to 49 mg. of protein per calorie. With respect to protein and energy content the human food value of natural cows' milk is therefore proportional to the amount of 4 per cent milk by the above formula. For the purpose of a usable feeding standard for milk production, the milk may be converted to a 4 per cent basis by the above energy formula and the digestible energy and protein requirements then expressed as constant multiples of the 4 per cent milk. It is suggested that feeding standards for milk production might be adequately handled by determining only the energy and protein in the feed, together with their digestion coefficients.

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# INHERITANCE OF REACTION TO STEM RUST AND BARBING OF AWNS IN BARLEY CROSSES<sup>1</sup>

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## INTRODUCTION

Information regarding the mode of inheritance of reaction to certain plant pathogenes aids the plant breeder in developing a logical plan for the improvement of particular crop plants. This paper presents the results of a study of the transmission of reaction to stem rust (*Puccinia graminis tritici* Eriks. and Henn.) in a cross between susceptible and resistant varieties of barley (*Hordeum vulgare* L.). The data were taken in an endeavor to determine the number of factor pairs differentiating resistance and susceptibility and whether these factors are inherited independently of the *Rr* factor pair for rough and smooth awn.

Considerable progress has been made in determining the manner in which resistance to the attacks of stem rust is inherited in the genus *Triticum*, but the writers have been unable to find references to work of this nature with the genus *Hordeum*. Since the literature pertaining to *Triticum* has recently been reviewed by Hayes et al.<sup>3</sup> such a review is not included here.

## MATERIALS AND METHODS

The parents used in the crosses were Glabron, Peatland, and Minnesota No. 462. Glabron and Minnesota 462 are smooth-awned sister selections from a cross of Smooth Awn × Manchuria. Peatland is a rough-awned selection from a variety called Switzerland.

Both the  $F_1$  and  $F_3$  generations were grown in 6-foot rows 1 foot apart, the  $F_1$  being spaced 4 inches apart within the row and the  $F_3$ , 3 inches. The Glabron and Peatland parents were sown in every tenth and eleventh row, respectively. The  $F_3$  families were from seed of  $F_2$  plants chosen at random.

No artificial epidemic of stem rust was attempted. The field nursery in which the barley was grown was 2 or 3 rods from the spring-wheat rust nursery. In the epidemic induced in spring wheat a large number of physiologic forms of stem rust were used. It is possible that most of the barley infection was a result of the spread of rust from the spring wheat.

In order to identify the physiologic forms present, urediospores were collected from the nearly mature plants of Glabron, Peatland,

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<sup>3</sup> HAYES, H. K., AUSEMUS, E. R., STAKMAN, E. C., and BAMBERG, R. H. CORRELATED INHERITANCE IN SPRING WHEAT CROSSES OF REACTION TO STEM RUST, LEAF RUST, BLACK CHAFF, AND BUNT. Unpublished manuscript.

Minnesota 462, the  $F_1$  generation of Minnesota 462  $\times$  Peatland, and susceptible and resistant plants of one of the  $F_3$  families of the Peatland  $\times$  Glabron cross.

All notes were taken in the field at that stage in the development of the plants when the heads were mature but the culms were still somewhat green. The plants were pulled and classified as to barbing of awns and reaction to stem rust. An attempt was made to separate the rough and smooth-awned plants on the basis of the segregation of the *Rr* factor pair disregarding the *Ss* factor pair (Griffiee (3)).<sup>4</sup> Likewise only two classes were used in separating the resistant and susceptible plants, since it was impossible to differentiate between the homozygous resistant and the heterozygous resistant individuals.

In analyzing the data, Fisher's (1) method of partitioning  $\chi^2$  into its components was used, and the values of  $P$  are taken from his table of  $\chi^2$ . Throughout the analyses a value of  $P=0.05$  was taken as the lower level of significance. Values of  $P$  equal to or below this level give odds equal to or greater than 19:1 that the deviations noted are not due to errors of random sampling. Such values are considered as statistically significant throughout this study.

## EXPERIMENTAL RESULTS

### PHYSIOLOGIC FORMS PRESENT

Identification of the physiologic forms of *Puccinia graminis tritici* showed that Nos. 17, 38, and 49 were present. Forms 38 and 49 were isolated from plants of Minnesota 462 and Peatland; Forms 17, 38, and 49 from Glabron, Form 38 from the  $F_1$  generation of Minnesota 462  $\times$  Peatland and the resistant  $F_3$  plant of Peatland  $\times$  Glabron, and Forms 17, 38, and 49 from the susceptible  $F_3$  plant of Peatland  $\times$  Glabron. It is of interest to note that the susceptible  $F_3$  plant of Peatland  $\times$  Glabron was infected with the same three physiologic forms as Glabron, whereas from the resistant  $F_1$  generation of Minnesota 462  $\times$  Peatland and the resistant  $F_3$  plant of Peatland  $\times$  Glabron Form 38 only was isolated.

It is probable that the infection was caused by Forms 17, 38, and 49. However, it should be kept in mind that Hayes and others (5, 6, 7), Harrington (4), Stakman (9), and Goulden and Neatby (2) have shown that mature plants of some varieties of wheat under field conditions possess resistance to many and perhaps all forms of *Puccinia graminis tritici*. On the other hand, it seems probable that physiologic forms of *P. graminis secalis* Eriks. and Henn. were not present, and therefore the present study may not be indicative of the reaction of the crosses and parents to this variety of stem rust.

### REACTION TO STEM RUST

An epidemic of stem rust developed on the parents and on the  $F_1$  and  $F_3$  generations. Sixty-three  $F_3$  families from  $F_2$  plants chosen at random were classified for breeding behavior into homozygous resistant, heterozygous, and homozygous susceptible. The accuracy of the classification was measured by the reaction of the Peatland and Glabron parents. Only one plant from the seven rows of the Peatland parent proved susceptible. It is believed that susceptibility in this instance was due to mechanical mixture of the seed, such as may occur in thresh-

<sup>4</sup> Reference is made by number (italic) to Literature Cited, p. 1129.

ing and planting operations. Two of the seven rows of the Glabron parent possessed resistant plants, the frequency in these two rows being 5.6 and 15.4 per cent. These apparently resistant plants probably were genetically susceptible ones which escaped infection by chance.

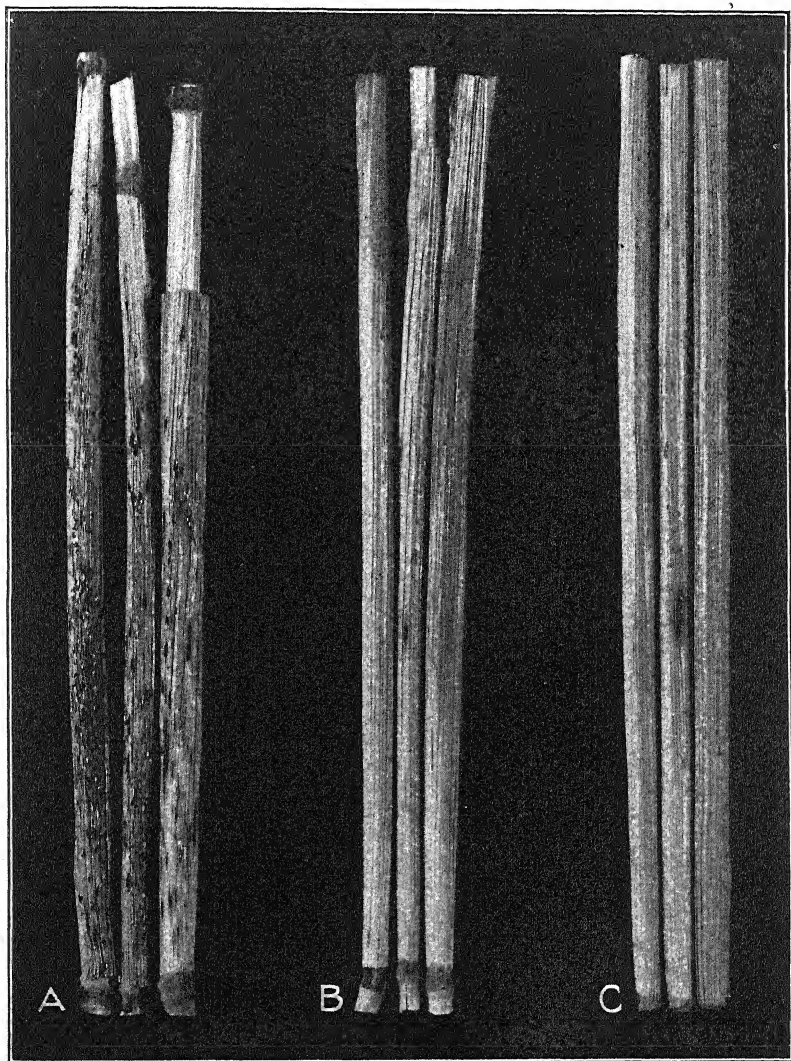


FIGURE 1.—Dominance of resistance to stem rust: A, Susceptible parent, Minnesota 462; B,  $F_1$  generation of a cross between Minnesota 462 and Peatland; C, resistant parent, Peatland

#### DOMINANCE

It was impossible to distinguish between the heterozygous and the homozygous resistant plants of the second generation, as Figure 1 shows, and therefore the plants were classified by means of the  $F_3$  families. The results obtained in the  $F_3$  generation are illustrated in Figure 2. The culms of Glabron are shown in Figure 2, A, those

from a homozygous susceptible  $F_3$  progeny in 2, B, those from a homozygous resistant  $F_3$  progeny in 2, C, and those of the Peatland parent in 2, D. As in  $F_2$ , the plants of the  $F_3$  could be placed only in the two classes, resistant and susceptible, since the heterozygous individuals were indistinguishable from those homozygous for resistance. It may be concluded, therefore, that in this cross resistance is dominant to susceptibility.

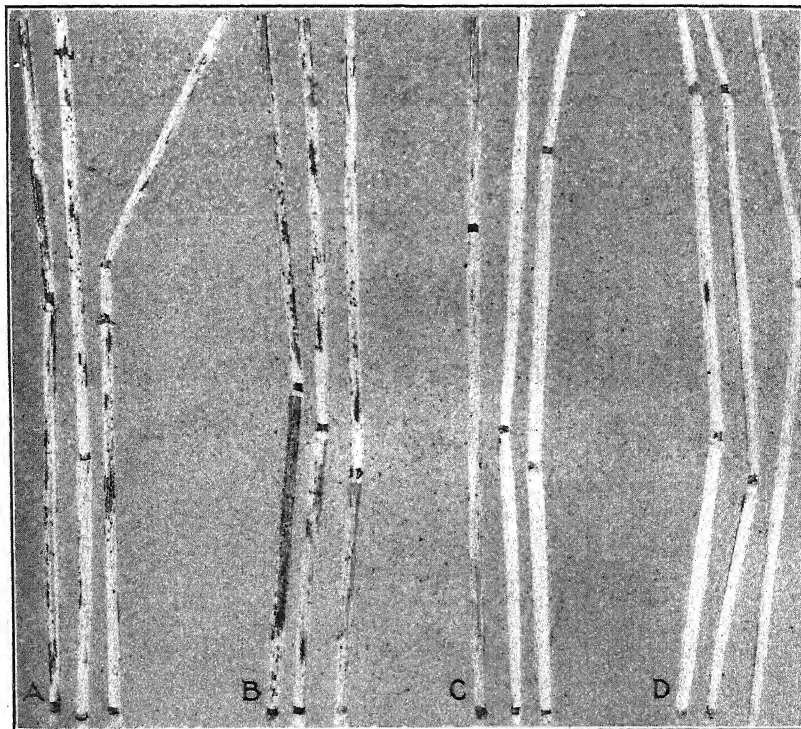


FIGURE 2.—Reaction to stem rust of parents and of a homozygous susceptible and a resistant  $F_3$  plant of a cross between Peatland and Glabron: A, Glabron, susceptible parent; B, susceptible  $F_3$  plant; C, resistant  $F_3$  plant; and D, Peatland, resistant parent

TABLE 1.—Segregation of the  $F_2$  generation of Peatland  $\times$  Glabron for reaction to stem rust, the classification being based upon the reaction of  $F_3$  families

Phenotypic class	Genotype	Number of families obtained	Number of families expected on basis of 1-factor pair difference
Homozygous resistant.....	$TT$	17	16
Heterozygous.....	$Tt$	32	31
Homozygous susceptible.....	$tt$	14	16

Table 1 presents the phenotypes and genotypes of the  $F_2$  generation of the cross Peatland  $\times$  Glabron as determined from the  $F_3$  families. Statistical analyses are not necessary to show that the numbers

obtained in the three phenotypic classes are very close to those expected on the assumption that Glabron and Peatland are differentiated by a single factor pair. In this connection it should be pointed out that 4 of the 14 families classed as homozygous susceptible had a few resistant plants. The frequency of these resistant plants varied from 5.3 per cent to 16.7 per cent. A reversal of dominance would be necessary to explain resistant plants among those classed as homozygous for susceptibility. However, since resistant plants were found also in two rows of Glabron, it seems probable that those found in the four families grown from susceptible parents are in reality plants that escaped infection and therefore the placing of these four families in the homozygous susceptible class is justifiable. If the alternative were accepted (of putting them in the heterozygous class) a total  $\chi^2$  value of 2.8413 with a value of  $P$  between 0.20 and 0.10 would be obtained. This value is not statistically significant and therefore the data may be considered as fitting the hypothesis, which assumes that only 1 factor pair is involved in the cross.

It remains to be determined whether segregation within the  $F_3$  families supports the results obtained with the  $F_2$  generation. As Table 1 shows, 32  $F_3$  families were found to be segregating for reaction to stem rust. These 32 families represented a total of 2,196 plants, of which 1,646 were resistant and 550 were susceptible. Statistical analysis is not necessary to demonstrate that this is very close to the ratio of 1,645 resistant:549 susceptible, which would be expected if resistance and susceptibility are differentiated by a single factor pair.

However, Kirk and Immer (8) have pointed out that a factorial analysis based upon the total number of plants in the different classes disregarding segregation within families is not always reliable, as inherent discrepancies may be covered up. To determine whether such were the case in these studies, the  $\chi^2$  test for goodness of fit was applied to the data. The results are shown in Table 2.

TABLE 2.— $\chi^2$  test for goodness of fit between the reaction of  $F_3$  families to stem rust and the theoretical expected based on a 1-factor pair difference

Family No.	Resistant	Susceptible	$\chi^2$	$P$ lies between—	Family No.	Resistant	Susceptible	$\chi^2$	$P$ lies between—
1537.....	59	25	1.0159	0.50 and 0.30	1585.....	12	5	0.1765	0.70 and 0.50
1540.....	60	21	1.0370	.90 and .80	1587.....	54	17	.0423	.90 and .80
1542.....	59	20	.0042	.95 and .90	1589.....	17	2	2.1228	.20 and .10
1543.....	67	25	.2319	.70 and .50	1590.....	13	5	.0741	.80 and .70
1546.....	54	27	2.5720	.20 and .10	1599.....	28	6	.9804	.50 and .30
1551.....	86	24	.5939	.50 and .30	1600.....	75	21	.5000	.50 and .30
1552.....	73	26	.0842	.80 and .70	1601.....	69	32	2.4059	.20 and .10
1554.....	82	32	.5731	.50 and .30	1604.....	30	12	.2857	.70 and .50
1557.....	29	12	.3984	.70 and .50	1606.....	7	2	.0370	.90 and .80
1564.....	70	21	1.1795	.70 and .50	1610.....	43	15	.0230	.90 and .80
1573.....	23	7	.0444	.90 and .80	1611.....	77	15	3.7101	.10 and .05
1574.....	32	17	3.5986	.10 and .05	1612.....	81	23	.4615	.50 and .30
1577.....	60	27	1.6897	.20 and .10	1615.....	27	12	.6923	.50 and .30
1578.....	26	15	2.9350	.10 and .05	1603.....	34	3	5.6306	.02 and .01
1579.....	89	21	2.0485	.20 and .10	1538.....	50	32	8.6016	.01 and .00
1581.....	76	17	2.2401	.20 and .10	1556.....	84	11	9.1263	.01 and .00

Total  $\chi^2$  value=53.1165.

Deviations of  $\chi^2=2.37 \times$  standard error.

An examination of Table 2 reveals the fact that the last 3 families gave values of  $P$  lower than 0.05. Due to the large values of  $\chi^2$  in these three families, the total  $\chi^2$  for all of the  $F_3$  families (53.1165) slightly exceeds the limits of significance. By Fisher's (1) method

the deviations noted are 2.37 times the standard error. Since only 3 of the 32 families deviated significantly from the expected, a test to determine whether the frequency distribution of  $\chi^2$  values for individual families fits the expected was made. The results of such a test, as outlined by Kirk and Immer (8) are given in Table 3.

TABLE 3.—Frequency distribution of  $\chi^2$  values for goodness of fit between the reaction of individual  $F_3$  families to stem rust and the theoretical expected based on a 1-factor pair difference

$\chi^2$	$P$	Expected	Obtained	$\frac{(O-C)^2}{C}$	$\chi^2$	$P$	Expected	Obtained	$\frac{(O-C)^2}{C}$
0.0000	1.00	0.32	1	1.5125	0.4550	0.50	6.40	7	0.0563
.0002	.99	.32			1.0740	.30	3.20	0	3.2000
.0006	.98	.96			1.6420	.20	3.20	6	2.4500
.0039	.95	1.60			2.7060	.10	1.60	3	.0001
.0158	.90	3.20	5	1.0125	3.8410	.05	.96		
.0642	.80	3.20	2	.4500	5.4120	.02	.32		
.1480	.70	6.40	5	.3063	6.6350	.01	.32		

$\chi^2 = 8.9877$ .

$P$  lies between 0.30 and 0.20.

Because of the small numbers, the upper four expected values and the lower four were each grouped into one class. The  $\chi^2$  value obtained was 8.9877, which has an accompanying  $P$  value between 0.30 and 0.20. Probably the three exceptional families may be accounted for by chance alone, and the segregation of  $F_3$  families for reaction to stem rust is that expected if the Glabron and Peatland parents differ by a single factor pair. Since the results from both the  $F_2$  and the  $F_3$  generations indicate that the parent varieties differ in their reaction to stem rust by a single factor pair, the genotype of Peatland is designated  $TT$  and that of Glabron  $tt$ . The letter  $T$  is taken from *tritici*, because the physiologic forms to which Peatland is resistant are classified as *Puccinia graminis tritici*.

#### INDEPENDENCE OF INHERITANCE OF REACTION TO STEM RUST AND BARBING OF AWNS

The results of studies to determine the linkage relationships between reaction to stem rust and barbing of awns are presented in two parts: (1) The evidence furnished by the genotypes of the  $F_2$  plants as determined by the breeding behavior of the  $F_3$  families, and (2) an analysis of the  $F_3$  families that segregated for both reaction to stem rust and barbing of awns.

##### EVIDENCE FURNISHED BY THE $F_2$ GENERATION GENOTYPES

To determine whether the  $F_2$  generation segregated in the ratio of 9:3:3:1, as would be expected if the  $RR$  and  $TT$  factor pairs are not linked, the  $\chi^2$  test for goodness of fit was applied to the data. A  $\chi^2$  value of 4.0758 was obtained, giving a value for  $P$  between 0.30 and 0.20. A more detailed study was made by applying the  $\chi^2$  test to a classification of the  $F_2$  generation based on the nine different genotypes expected. The results, summarized in Table 4, show 31 rough-awned and resistant, 10 rough-awned and susceptible, 18 smooth-awned and resistant, 4 smooth-awned and susceptible, which is very close to the ratio 32:9:17:5, which would be expected on the basis of independent inheritance. A  $P$  value lying between 0.10 and 0.05 was obtained. Considering that there were only 63  $F_3$  families on which to classify the  $F_2$  generation, the fit between the expected and the obtained is good.

So far as the  $F_2$  generation is concerned, therefore, the evidence sustains the hypothesis that reaction to stem rust and barbing of awns are independently inherited.

TABLE 4.— $\chi^2$  for goodness of fit to test whether the number of individuals obtained in the nine different  $F_2$  generation genotypic classes vary significantly from the expected

Genotype	Phenotype	Obtained	Expected	$\frac{(O-C)^2}{C}$
<i>RRTT</i> .....	Rough awn and resistant.....	2	3.9375	0.9534
<i>RRtt</i> .....	do.....	13	7.8750	3.3353
<i>RrTT</i> .....	do.....	7	7.8750	.0972
<i>RrTt</i> .....	do.....	9	15.7500	2.8929
<i>Rrtt</i> .....	Rough awn and susceptible.....	1	3.9375	2.1915
<i>Rrtt</i> .....	do.....	9	7.8750	.1907
<i>rrTT</i> .....	Smooth awn and resistant.....	8	3.9375	4.1915
<i>rrTt</i> .....	do.....	10	7.8750	.5734
<i>rrtt</i> .....	Smooth awn and susceptible.....	4	3.9375	.0010

$\chi^2=14.3969$ .

$P$  lies between 0.10 and 0.05.

#### EVIDENCE FURNISHED BY ANALYSIS OF $F_3$ FAMILIES

An analysis of the  $F_3$  families segregating for both characters permits a study of considerably larger numbers of plants (767) than the  $F_2$ ; hence the results are more reliable.

In studying the  $F_3$  data it is well to keep in mind that should there be linkage between *RR* and *TT* due to crossing over in the  $F_1$ , some of the  $F_3$  families would represent the coupling phase and others the repulsion phase. Since the original cross was in the coupling phase the closer the linkage the greater should be the preponderance of families in this phase. As both the coupling and repulsion phases may be present it seemed desirable to apply to each  $F_3$  family a  $\chi^2$  test for goodness of fit to a 9:3:3:1 ratio. When this was done it was found that 4 of the 10 families segregating for both factor pairs deviated significantly from the theoretically expected ratio of 9:3:3:1. (Table 5.)

TABLE 5.— $\chi^2$  for goodness of fit to a 9:3:3:1 ratio of those  $F_3$  families segregating for barbing of awns and reaction to stem rust, partitioned into its components<sup>1</sup>

Family No.	Ratio obtained	$\chi^2$ for goodness of fit to a 9:3:3:1 ratio	$\chi^2$ for goodness of fit to a 3:1 ratio		Interaction between type of awn and reaction to stem rust
			Rough v. smooth	Resistant v. susceptible	
1537.....	37:17:22:8	6.2439	5.1429	1.0159	0.0851
1579.....	64:18:25:3	3.5192	.0121	2.0485	1.4586
1585.....	9:3:3:2	.8824	.1765	.1765	.5294
1581.....	64:14:12:3	6.3453	3.9032	2.2401	.2020
1601.....	50:21:19:11	4.5226	1.1914	2.4059	.9253
1610.....	35:14:8:1	3.7318	2.7816	.0230	.9272
1543.....	62:21:5:4	12.0773	11.3623	.2319	.4831
1556.....	79:8:5:3	28.3029	13.9263	9.1263	6.2503
1573.....	11:4:12:3	10.4148	10.0000	.0444	.3704
1577.....	54:25:6:2	13.8429	11.5900	1.6897	.5632
Total.....		89.8831	60.0863	19.0022	10.7946

<sup>1</sup> Levels of significance (Fisher (*l*))  $n=1$  is 3.841,  $n=10$  is 18.307,  $n=30$  is 43.773.



These results made it expedient to analyze the  $F_3$  families further. This was accomplished by partitioning  $\chi^2$  into its components. The method employed was similar to that outlined by Fisher (1) for back-cross data. Since the ratio of rough to smooth type of awn is entirely independent of the ratio of resistant to susceptible plants, that part of the deviations not due to segregation for barbing of awn and for reaction to stem rust must be due to linkage between  $Rr$  and  $Tt$ .

Tests for goodness of fit to a 3:1 ratio for segregation of barbing of awns of these 10  $F_3$  families were made by means of  $\chi^2$ . Likewise tests for goodness of fit to a 3:1 ratio for segregation of  $F_3$  families into resistant and susceptible classes were made. The interaction between type of awns and stem rust was obtained by subtracting the  $\chi^2$  due to segregation for barbing of awns plus the  $\chi^2$  due to segregation for reaction to stem rust from the total  $\chi^2$  of each  $F_3$  families for goodness of fit to a 9:3:3:1 ratio. The values obtained for the interaction between type of awn and reaction to stem rust should approximate those obtained from a  $\chi^2$  test for independent inheritance.

Table 5 shows that with the exception of family 1556 all of the significant  $\chi^2$  values are found in the column headed "Rough *v.* smooth." The segregation is definitely not that to be expected on the basis of a 1-factor pair difference between rough and smooth-awned types. Since the classification for barbing of awns was made without the aid of the binoculars it is believed that the deviations noted in five of the families are due to the  $Ss$  factor pair (Griffiee (3)), which also affects barbing of awns. A total value of 10.7946 was obtained for the interaction between type of awn and reaction to stem rust. This value is not significant, and therefore, except possibly in the case of family 1556, it does not seem that linkage is responsible for the  $\chi^2$  for goodness of fit of  $F_3$  progenies to a 9:3:3:1 ratio being significant. The method employed of determining whether two characters are independently inherited is superior to the  $\chi^2$  test for independence since considerably more information may be derived from the same amount of data.

The data for the  $F_2$  and  $F_3$  generations support the hypothesis that in the cross between Peatland and Glabron reaction to stem rust and barbing of awns are inherited independently of each other.

#### SUMMARY AND CONCLUSION

Inheritance of resistance to stem rust of wheat and barbing of awns was studied in crosses of barley.

Identification studies showed that the physiologic forms of *Puccinia graminis tritici* causing the stem rust were Nos. 17, 38, and 49.

Resistance was found to be dominant to susceptibility.

The reaction to stem rust of Peatland, a resistant parent, and Glabron, a susceptible parent, was differentiated by a single factor pair. This factor pair was designated  $Tt$ .

An analysis of  $F_2$  genotypes and of 10  $F_3$  families segregating for barbing of awns ( $Rr$ ) and reaction to stem rust ( $Tt$ ) showed that these two factor pairs are not linked.

Since reaction to stem rust is differentiated by a single factor pair and is not genetically linked with barbing of awns, it should be relatively easy to produce a smooth-awned, high yielding variety of barley resistant to physiologic forms of stem rust Nos. 17, 38, and 49



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# LABORATORY TESTS WITH VARIOUS FUMIGANTS ON CODLING-MOTH LARVAE<sup>1</sup>

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## INTRODUCTION

The rapid increase in codling-moth infestation from year to year in the aging apple orchards of New Jersey and other States offers a difficult problem to the orchardist. The standard spray of lead arsenate often fails to give efficient control of this pest because of the overwhelming numbers of larvae appearing during the hatching periods of the first and second broods. More efficient control has been obtained by the addition of white-oil emulsion or fish oil to the lead arsenate, the oil acting as a sticker for the lead arsenate as well as an ovicide for the codling-moth eggs. The number of summer oil sprays, however, which can be used without injury to apple trees is limited to about 2 or at most 3, during 1 season. Even with this limited number of oil sprays, injury to foliage and fruit has been observed (1, 5)<sup>2</sup> in New Jersey. While searching for other means of controlling this pest, Dr. T. J. Headlee considered the possibility of fumigating apple trees in the dormant stage with liquid hydrocyanic acid or other suitable fumigants. Following his suggestion, the writer carried out fumigation tests in the laboratory with some of the outstanding fumigants in order to determine their toxicity to codling-moth larvae. The results are presented in this paper.

## DESCRIPTION OF FUMIGANTS USED

Hydrocyanic acid (HCN) in liquid form is colorless, miscible with water in all proportions, boils at 26.5° C., and has a specific gravity of 0.702 at 16°. Its vapors are very volatile, inflammable, and extremely poisonous to man. Great precaution should be observed in handling it. Since 1916 it has been extensively used (2, 3, 6, 7, 8) in fumigating citrus trees against scale.

Ethylene chlorhydrin ( $C_2H_4OCl_2$ ) is a colorless liquid, soluble in water, possesses an ethereal odor, boils at 128° C. and has a specific gravity of 1.213 at 20°.

Ethylene dichloride ( $C_2H_4Cl_2$ ) is a colorless liquid with chloroformlike odor, insoluble in water, boils at 83.5° C., and has a specific gravity of 1.254 at 20°. The two aforementioned compounds were tested by Roark and Cotton (4).

Ethylene oxide ( $C_2H_4O$ ) is a colorless liquid, boiling at 14° C., gaseous at room temperature, soluble in water, and has a specific gravity of 0.887 at 7°. The concentrated vapors are inflammable but practically noninjurious to man. The fumigating value of this compound was first reported by Roark and Cotton (4).

Diethylene oxide ( $C_2H_4O$ )<sub>2</sub> is a colorless, volatile liquid, boils at 101° C., has a specific gravity of 1.037 at 20°, and is soluble in water in all proportions.

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<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 1136.

Ethyl acetate ( $C_4H_8O_2$ ) is a colorless liquid of strong ethereal odor, soluble in water, boils at  $77^\circ C.$ , and has a specific gravity of 0.9 at  $20^\circ$ . Vapors of ethyl acetate were found by Roark and Cotton (4) to be toxic against grain weevil.

Carbon bisulphide ( $CS_2$ ) is a colorless liquid of 1.29 specific gravity. Its vapor is 2.5 times as heavy as air and highly explosive when mixed with 3 times its volume of oxygen. It boils at  $46.1^\circ C.$ , and is only slightly soluble in water. It possesses high toxicity to insects, both in adult and larval stages.

### EXPERIMENTAL METHODS

All the chemicals tested were applied in liquid form and were allowed to vaporize in the fumigating chamber. The insects, in numbers of 20 or more, were placed in wire cages and transferred to the fumigating chambers. The chemicals were then added by the use of a pipette, and the chamber was tightly closed. After 1 hour the insects were removed from the chamber and kept for 24 hours in the laboratory before the final observations were made.

A period of 24 hours for final observations was found necessary in order to avoid erroneous results. Preliminary tests showed that some larvae, appearing dead immediately after the test, partially recovered after several hours exposure to the air. This behavior was especially noticeable when hydrocyanic acid was used as the fumigant. On the other hand, some larvae, which were alive when removed from the fumigating chamber, died a few hours later. The mortality was especially pronounced when ethylene chlorhydrin was used.

For all the chemicals tested, except the liquid hydrocyanic acid, large glass bottles of approximately 20-l capacity (19,750 cc) were used as fumigating chambers. The tests were carried out in the following manner: Wire cages containing the insects were placed in the glass container. The chemicals were applied from a pipette on strips of cheesecloth suspended from rubber stoppers. The cheesecloth absorbed and spread the chemical over a large surface, thus aiding in rapid vaporization. The bottle was then tightly stoppered and allowed to stand for 1 hour, with frequent shaking to produce equal distribution of the vapors. After 1 hour the cages were removed and exposed to laboratory temperature for 24 hours. The insects were then removed and examined. The bottles were thoroughly aerated between tests.

A special fumigating chamber was prepared for the hydrocyanic acid on account of the danger in handling it. This consisted of a round container, having a capacity of 5 cubic feet and made of block tin. An opening about 3 inches in diameter, made airtight by the aid of a top screw and lid, was provided on the top for introducing the insects and fumigant. Two small openings fitted with stopcocks, one on the top and one on the side near the bottom, were used for aerating the chamber after each test. In the tests with hydrocyanic acid gas the cages containing the insects were first transferred to the fumigating chamber. A small sheet of asbestos, heated to about  $100^\circ C.$  was then placed in the chamber for the purpose of causing rapid evolution of the gas, and the desired amount of liquid hydrocyanic acid was transferred from a pipette (especially prepared for this purpose) to the hot sheet. The upper opening was closed quickly and made airtight. In order to produce an equal distribution of the gas the container was shaken several times during the period of testing. At the end of this period the chamber was thoroughly aerated by

passing air under 30 pounds pressure through it for 5 to 10 minutes. The insects were then removed, transferred to new cages, and kept in the laboratory for 24 hours when the final observations were made.

By this method seven of the outstanding fumigants were tested on codling-moth larvae, both "naked" and in hibernacula. The larvae were obtained from either burlap or corrugated paper bands removed from apple trees. For the "naked" tests the larvae were completely freed from their hibernacula before being transferred to the cages. The number of larvae used in each test varied from 50 to 200.

The minimum dosages of the various fumigants, calculated in cubic centimeters of liquid per 100 cubic feet of space, required to kill 75 per cent or more of the larvae at laboratory temperatures were first determined. A comparison of the data in Table 1 shows that ethylene chlorhydrin and hydrocyanic acid proved most toxic, with minimum dosages of 20 and 30 cc, respectively. Carbon bisulphide and ethyl acetate ranked very low in toxicity, with minimum dosages of 286 and 572 cc, respectively. Diethylene oxide, ethylene oxide, and ethylene dichloride were midway between the two extremes, with minimum dosages of 115, 143, and 215 cc, respectively.

TABLE 1.—Minimum dosages of various fumigants required to kill 75 per cent or more codling-moth larvae at laboratory temperatures

Fumigant used	Chemical formula	Temperature (°F.)	Relative humid- ity	Quantity used per 100 cubic feet	Total larvae tested	Dead after 24 hours	
						Naked	In hiber- nacula
			Per cent	cc	Number	Per cent	Per cent
Ethylene chlorhydrin.....	C <sub>2</sub> H <sub>5</sub> OCl.....	76	51	20	100	75	75
		76	51	36	100	-----	78
Hydrocyanic acid.....	HCN.....	68	61	30	200	90	-----
		68	61	60	200	-----	78
Ethylene oxide.....	C <sub>2</sub> H <sub>4</sub> O.....	70	40	143	50	90	-----
		70	40	572	50	-----	100
Ethylene dichloride.....	C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub> .....	75	30	215	50	80	-----
		75	30	286	50	-----	76
Diethylene oxide.....	(C <sub>2</sub> H <sub>4</sub> ) <sub>2</sub> O.....	74	50	115	50	92	-----
		74	30	286	100	80	-----
Carbon bisulphide.....	CS <sub>2</sub> .....	74	30	572	50	-----	91
		76	32	572	50	80	-----
Ethyl acetate.....	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub> .....	76	32	1,174	50	-----	78
		-----	-----	-----	100	2	-----
None (check).....	-----	-----	-----	-----	100	-----	None

These results indicate that ethylene chlorhydrin and hydrocyanic acid gas are very toxic to codling-moth larvae. It was, therefore, deemed advisable to test these two compounds further at various temperatures. Three sets of temperatures were selected as follows: 40° F., or lower; 50° to 60°; and 60°, or higher. For the two lower sets of temperatures advantage was taken of the exceptionally mild fall of 1931, and the tests were carried out in an open insectary. During the latter part of November and the early part of December the morning temperatures were just above freezing and gradually rose during the day to about 60° or higher. Tests under the higher set of temperatures were carried out in the laboratory.

TABLE 2.—Tests with ethylene chlorhydrin on naked codling-moth larvae and larvae in the hibernacula fumigated for 1 hour at different temperatures

## NAKED LARVAE

Temperature (°F.)	Relative humid- ity	Fumi- gant used per 100 cubic feet	Total larvae tested	Larvae dead after 24 hours
	<i>Per cent</i>	<i>cc</i>	<i>Number</i>	<i>Per cent</i>
68-72	32-51	10	60	62
		20	100	83
		30	50	100
		70	50	100
		140	50	100
50-56	50	10	60	60
		20	50	72
		40	50	100
		70	60	100
		140	50	100
37-40	50	20	50	6
		40	50	28
		70	50	100
		140	50	100

## LARVAE IN HIBERNACULA

54-62	29-46	20	50	40
		30	50	53
		40	50	100
		70	60	100
		140	60	100
37-40	30-56	20	60	0
		40	60	0
		70	60	94
		140	60	100

## TOXICITY OF ETHYLENE CHLORHYDRIN AT DIFFERENT TEMPERATURES

The results on naked larvae, presented in table 2, indicate that the toxicity of ethylene chlorhydrin is not changed at temperatures varying from 50° to 72° F. At this wide range of temperatures 30 cc per 100 cubic feet was the minimum dosage required to kill 100 per cent larvae. At temperatures of 40° or lower, the toxicity of ethylene chlorhydrin was decidedly decreased, a dosage of 70 cc per 100 cubic feet being required to produce 100 per cent kill. When tested on larvae in hibernacula (Table 2) a dosage of 40 cc per 100 cubic feet of space was sufficient to produce 100 per cent kill, at temperatures of 54° to 62°, whereas more than three times this quantity (140 cc) was required to kill 100 per cent of the larvae at temperatures of 37° to 40°.

## TOXICITY OF HYDROCYANIC ACID GAS AT DIFFERENT TEMPERATURES

Results similar to those obtained with ethylene chlorhydrin were also obtained with hydrocyanic acid gas (Table 3). Tests on naked larvae showed no differences in toxicity between temperatures of 54° to 72° F., 40 cc per 100 cubic feet being the minimum dosage required to kill from 90 to 100 per cent of the larvae. At a temperature of 40°, or lower, however, the toxicity was greatly reduced and 80 cc of the gas gave only about 30 per cent kill. When tested on larvae in hibernacula, no appreciable differences in toxicity were observed at temperatures ranging from 54° to 64°, a dosage of 30 cc per

100 cubic feet giving 92 to 100 per cent kill of larvae. On the other, hand, at 40° or lower a dosage of 80 cc gave 30 per cent kill, whereas a dosage of 160 cc killed only 44 per cent of the larvae.

TABLE 3.—Tests with hydrocyanic acid gas on naked codling moth larvae, and larvae in the hibernacula, fumigated for 1 hour at different temperatures

NAKED LARVAE

Volume of fumigant used per 100 cubic feet	Total of larvae tested	Larvae dead after 24 hours	Temperature (°F.)	Relative humidity
Cc	Number	Per cent		Per cent
20	50	57	64-72	48-60
40	100	96		
80	100	100		
20	100	64		
40	100	98	54-56	90
80	100	100		90
20	100	20		46
40	100	36	34-40	79
80	100	30		82
40	100	63		63
40	100	42	60-62	61
80	200	96	48-52	58
80	200	100	58-60	80
80	100	92	54	54
40	100	22	36-40	56
80	100	30		75
160	100	44		72
				68

The results presented in the tables clearly indicate that at a temperature of 50° F., or higher, both hydrocyanic acid and ethylene chlorhydrin are more toxic to codling-moth larvae than at a temperature of 40°, or lower. These differences may be explained in two ways: (1) Both fumigants volatilize very slowly at low temperatures, and not enough gas accumulates within 1 hour to produce a lethal concentration. Even where the evolution of the hydrocyanic acid gas was hastened by the aid of a hot asbestos sheet, the low temperature in the fumigating chamber would tend to condense the gas back into its liquid form and thus decrease its rates of diffusion. (2) At lower temperatures the insects are less active than at higher temperatures and may not be so readily susceptible to the poisonous action of the fumigants.

SUMMARY

The following fumigants were tested on codling-moth larvae at different temperatures under laboratory conditions: Liquid hydrocyanic acid, ethylene chlorhydrin, carbon bisulphide, ethyl acetate, ethylene oxide, diethylene oxide, and ethylene dichloride. The minimum dosages, calculated in cubic centimeter of liquid per 100 cubic feet of space, required to kill 75 per cent or more of larvae were determined.

The results from tests on naked larvae show that ethylene chlorhydrin and hydrocyanic acid proved most toxic, the minimum dosages being 20 cc and 30 cc, respectively. Carbon bisulphide and ethyl acetate ranked very low in toxicity, the minimum dosages being 286 cc and 572 cc, respectively. Diethylene oxide, ethylene oxide, and ethylene dichloride were midway between the two extremes, with

minimum dosages of 115, 143, and 215 cc, respectively. The tests on larvae in hibernacula show, in general, the same order of toxicity for the various fumigants as observed on naked larvae, with the exception that the minimum dosages were considerably larger. Low temperatures greatly decreased the percentage of kill obtained with both hydrocyanic acid and ethylene chlorhydrin.

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# APPARATUS FOR MAKING AUTOGRAPHIC RECORDS OF CATALASE ACTIVITY OF PLANT TISSUES, AND THE PROCEDURE INVOLVED<sup>1</sup>

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## INTRODUCTION

Variations in activity of the enzyme catalase, which is universally present in living tissue, may be a useful indicator of certain metabolic conditions of plants and animals. In the case of apple-tree tissues, for example, catalase activity of the leaves or of the bark affords a convenient and highly sensitive index to some changes in the internal condition of the plant, which in turn may be related to such manifestations as vigor of shoot growth, flower-bud formation, condition of maturity, resistance to winter injury, and the like. Similar relations between catalase and certain growth phenomena are found in other plants and tissues, including seeds and fruits (1, 2, 3, 6, 7, 8).<sup>2</sup>

While the determination of catalase activity is relatively simple, there is no widely adopted standard procedure. The results obtained by different workers depend to a large extent upon the methods used. The apparatus described in this article is an elaboration of the device illustrated in Cornell Memoir 74 (4). Its chief features are that it enables the simultaneous determination of catalase activity in 12 different samples of tissue with an autographic record of the results in each case. In using this equipment there is a great saving of time wherever numerous determinations are to be made at frequent intervals. The graphic records afford an accurate and convenient means of comparing the catalase activity of different tissues or of the same tissue at different seasons.

## DESCRIPTION OF APPARATUS

Figure 1 shows the complete apparatus with the 12 reaction chambers in use. The 2-arm reaction tubes (*k*) and the gas-collecting bulbs (*l*) are immersed in a copper water bath (*c*), which normally occupies the position outlined by the dashes. The bath is kept at a constant temperature by means of an electric heating unit controlled by a thermoregulator. The motion of the shaker (*a*) agitates the water, thereby insuring a uniform temperature throughout.

The shaking device, which also supports the reaction tubes, is made of brass and is operated by an electric motor. The rate of vibration of the shaker and the distance to and fro may be governed by varying the diameter of the pulley and the length of the levers. (Fig. 1, *a'*.) When the tubes are held on the support by the spring clamps they must be tilted forward. Such a position provides a

<sup>1</sup> Received for publication Dec. 12, 1932; issued July, 1933.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 1143.

greater exposure of surface of the reacting solutions when the shaker is in operation. It is thus possible to provide thorough mixing of the liquids in the reaction tubes without the accumulation of all material alternately in one arm or the other. This insures the free release of oxygen at a regular rate.

The gas-collecting bulbs are held firmly in place by means of a special brass support. (Figs. 1, *b* and 2, *b*.) This support may be moved along rods which are fastened to the side of the water bath. (Fig. 1, *b'*.) Each gas bulb is connected to a reaction chamber by means of heavy rubber tubing. The rubber tube is just long enough to permit free motion of the shaker.

The details of connecting reaction tubes, gas bulbs, and burettes are clearly shown in Figure 1 and are indicated diagrammatically in Figure 2. The water, which is forced out of the gas bulbs (fig. 2, *a*, *b*)

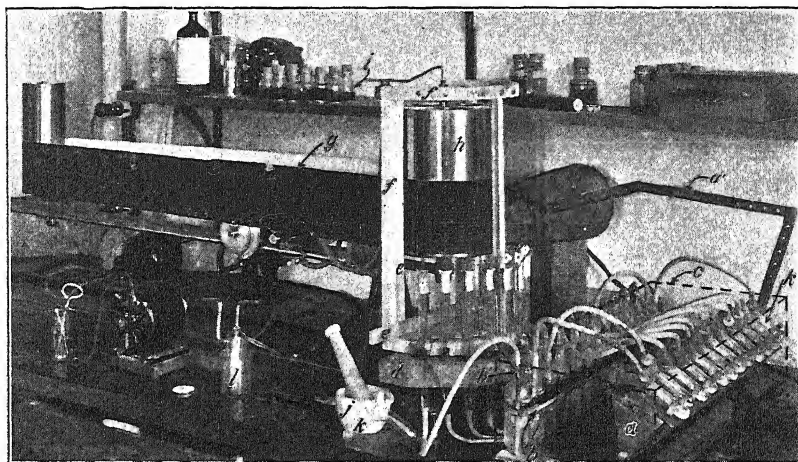


FIGURE 1.—General view of apparatus used for autographic records of catalase activity: *a*, *a'*, Brass shaking device and support for reaction chambers; *b*, *b'*, brass support for gas-collecting bulbs; *c*, position of constant temperature bath; *d*, base of wooden support upon which are mounted the burettes with floating indicator pens; *e*, movable support with guide wires by means of which pens may be held away from smoked paper; *f*, support for plumb lines, with spiral weight, which hold pens against smoked paper; *g*, smoked paper for autographic record of determination; *h*, revolving drum of kymograph; *i*, small bottles containing macerated tissue with prepared chalk and distilled water added; *j*, small mortar and large pestle used in triturated tissue; *k*, 2-arm tubes; *l*, gas-collecting bulbs used as reaction chambers

as the volume of oxygen increases as a result of the catalase reaction, is transferred to the glass tubes or burettes (fig. 2, *A*, *e*) containing the floating indicator pen. (Fig. 2, *A*, *f*.) These burettes are about 12 mm in internal diameter and are selected for uniformity of bore. They are firmly mounted with rubber gaskets in holes drilled at right angles in the thick wooden support. (Figs. 2, *A*, *d* and 2, *C*, *a*.) This support may be adjusted in any direction so as to permit proper leveling and alignment. The indicator pen in each tube is floated by a cork (fig. 2, *A*, *g*), saturated with paraffin, and is held in an upright position by the metal cap. (Fig. 2, *A*, *h*.) The indicator is made of stiff wire and consists of an upright member and a horizontal arm (fig. 2, *A*, *i*) whose length and position may be adjusted by moving the wires through the holes in the diamond-shaped metal union.

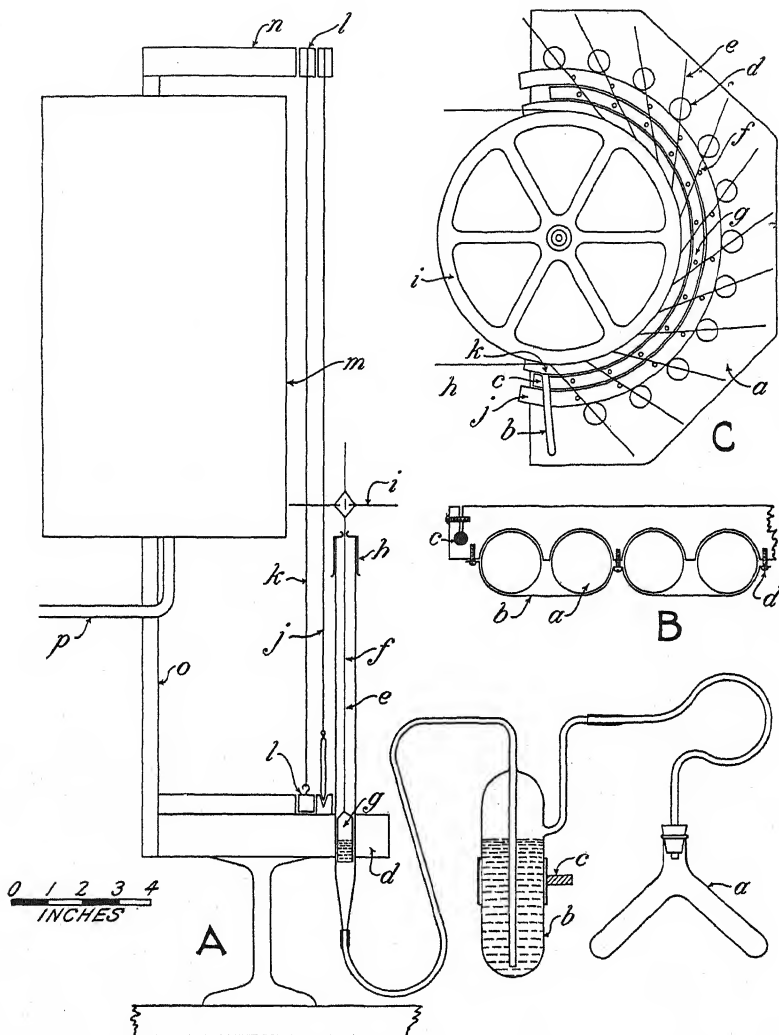


FIGURE 2.—A, Diagram of apparatus for autographic records of catalase determination, showing connections and longitudinal section of recording device: *a*, Reaction tubes, capacity of each arm about 10 cc and total volume 40 cc; *b*, gas-collecting bulbs containing water, connected with *a* by heavy rubber tubing; *c*, cross section of brass support (top view shown in B); *d*, support for burettes and recording device; *e*, glass burette connected with *b* by heavy rubber tube; *f*, floating pen; *g*, cork float impregnated with paraffin; *h*, metal cap to guide indicator; *i*, horizontal arm which may be adjusted in any direction by moving through the small diamond-shaped metal connection plate; *j*, plumb line of fine wire with lead weight to hold pen against drum; *k*, taut wire stretched on support to hold pens away from drum; *l*, movable support for holding taut guide wires; *m*, drum of kymograph; *n*, upper part of support; *p*, support for drum. B, Brass support for holding gas-collecting bulbs: *a*, Cross section of bulb; *b*, metal tape; *c*, rod along which support may be moved up or down; *d*, screws for adjusting pressure of tape. C, Arrangement of indicating units as viewed from above: *a*, Base of heavy wooden support; *b*, lever for operating support; *c*, support which moves in track between *j* and *k*; *d*, cross section of burettes; *e*, horizontal arm of floating pen which moves vertically with change in level of liquid in burettes; *f*, plumb lines serving as guide wire to hold pen against drum; *g*, taut wires attached to movable support which hold pens away from drum when lever is pulled to left of position shown in diagram; *h*, smoked paper; *i*, revolving drum

When in operation, the horizontal arm of the indicator is held against the paper on the recording drum (fig. 2, A, *m*) by means of No. 36 constantan wire (fig. 2, A, *j*) which is suspended from the support (figs. 1, *f*, and 2, A, *n*). This wire is held taut by the lead weight attached to the lower free end. Care must be taken in suspending these plumb lines so that the pressure exerted against the arms is not great enough to interfere with the vertical movement of the indicator as it is forced up by the float. In the plan shown in figure 2, C, the small dots represent the points at which the plumb lines come in contact with the pens.

When not in use, the arms of the floating pens are held away from the drum by means of the guide wires (figs. 2, A, *k*, and 2, C, *g*) attached at the upper and lower ends of that part of the support which moves in a semi-circular groove and which is operated by a lever (figs. 2, A, *l*, and 2, C, *b* and *c*).

The smoked paper upon which the catalase activity of the sample is graphically recorded is fixed to the revolving drum of the kymograph. (Fig. 1, *g* and *h*.) The horizontal lines on the paper represent cubic centimeters of oxygen developed during the reaction. In calibrating the burettes to determine the exact spacing of these lines, successive cubic centimeters of air are added to the reaction tube through a 2-hole stopper substituted for that purpose. It is of course easy to determine whether the various units give comparable results. When equal amounts of the same solution are used in each of the 12 tubes, the curves obtained are identical in a proper set-up under standard conditions.

The kymograph is mounted on a grooved steel track, fastened to a heavy table. It may be pulled away from the support containing the indicating devices whenever the smoked paper is to be changed. The drum is operated by an electric motor and may be run at a wide range of speeds. Active as well as inactive samples may therefore be accommodated without taking up too much space of the chart. The full length of the chart should accommodate 8 sets each of 12 determinations, or 96 separate graphical records. The actual running time is recorded by means of a stop watch so that the graph may be translated in terms of volume and time. The running time, nature of sample, and any other pertinent data may be written on the chart, and the entire record may be rendered permanent by dipping the chart in varnish.

#### STANDARD PROCEDURE

In using the equipment described above, the following procedure has been found convenient for studies involving fruit-tree tissues and is the standard method used in work previously reported by the author (4, 5). The amount of tissue involved need not exceed 0.1 g fresh weight. This is furnished, for example, by 5 to 10 buds, by 20 disks of leaf tissue each 7 mm in diameter, or by 3 to 5 disks of bark each 5 mm in diameter. Larger amounts of tissue may, of course be used to obtain composite samples or for other purposes. A sharp cork borer is well adapted for sampling such tissues as leaves or bark. Unavoidable injury in sampling, such as occurs in cutting the bits of tissue, should be kept at a minimum and should be uniform.

The fresh tissue is held in 100-cc tightly stoppered bottles and is weighed as soon as possible after collection. After the sample is weighed, it is placed in a small mortar and moistened with about 1 to 2 cc of distilled water. Enough precipitated chalk is added to form a creamy mixture with which the tissue is thoroughly coated before trituration begins. It has been demonstrated that catalase activity may be greatly inhibited in some preparations unless neutralization occurs as soon as the cells are ruptured. In this connection it should be pointed out that the precipitated chalk is in a much finer state of division than are the ordinary samples of chemically pure powdered calcium carbonate, and probably for this reason the neutralization seems to be more effective in the former case. After thorough trituration with a large pestle (a bit of quartz being added when woody tissues are involved), enough distilled water is added to give a final dilution of 1 g of fresh material in 100 cc of water for active tissue, such as leaves or buds, and 1 to 50 for less active samples, such as bark or wood. The diluted preparations are returned to the small bottles and kept in a cool place.

In order to allow the preparation to come to equilibrium, determinations of catalase activity are usually made several hours after trituration of the tissue. Shorter or longer intervals may, of course, be adopted as standard. The sample used in each determination consists of 1 cc of the thoroughly mixed preparation. This is withdrawn quickly from the bottle with a 1-cc pipette before settling of the agitated suspension can take place. The tip of the pipette may be broken off to facilitate transfer to the suspension. Samples are placed in one arm of each of the 12 tubes which are clamped in place on the support and shaker. Into the other arm of each reaction tube 2 cc of neutralized 12-volume dioxogen is introduced. The stoppers provided with a glass outlet are then firmly fixed to the reaction chambers. Next, the rubber tubing, which remains fastened to the gas bulb, is connected to the stopper and is made to overlap from 3 to 5 cm on the glass outlet. This variation in the length of the connecting tube makes it possible to apply the exact pressure required to bring the arm of the indicator float on the level of the lowest line on the smoked paper. The level of the horizontal arm may itself be adjusted if need be. During these operations the sample and the dioxogen reach the temperature of the bath which is usually held at 20° C. The volume of air in the rubber tubing and above the liquids in the reaction chamber and gas collecting bulb is approximately 75 cc.

When all tubes are connected, the drum is set in motion and the shaker is started as soon as the smoked paper has reached a definite point at which the record of the determination is to begin. A stop watch is used to time the determination from the moment the mixing of dioxogen and the preparation first takes place.

The reaction may be allowed to continue for any desired length of time, usually between 100 and 600 seconds. At the end of a fixed period the horizontal arms of the pen may either be withdrawn from the paper by means of the lever operating the movable support with guide wires, or the drum may be stopped at that time, allowing the further development of gas to be recorded by the height of the vertical line.

After the determination is finished, the reaction tubes are disconnected, washed immediately, and drained thoroughly before they are again placed on the support for the next run. Duplicate or triplicate determinations of each sample can easily be made since only a small quantity of the preparation is required at one time.

Several typical graphs in which variations in catalase activity of apple-tree tissues are clearly shown are reproduced in Figure 3. It should be noted that one set of determinations shown on this figure

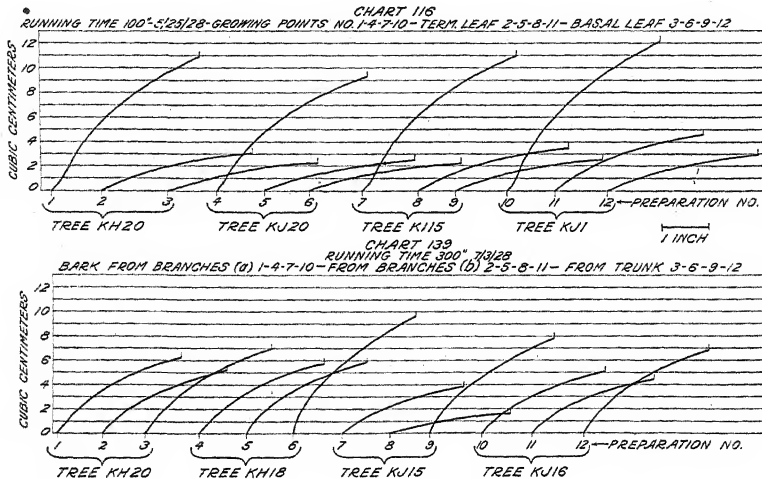


FIGURE 3.—Copies of autographic records of catalase activity. The records on chart 116 are for a run of 100 seconds, and on chart 139, for 300 seconds. Note variation in activity of different tissues and of the same tissue from different trees

lasted for only 100 seconds, the other for 300 seconds. It is obvious that the growing points are far more active than leaf tissue and that bark is less active than either. That the activity for a given time varies with the tree is also clearly shown. By measuring the distance the paper has traveled during a given time, it is easy to translate the results in terms of seconds required to develop a given amount of oxygen. The total amount of gas developed during the entire period is represented by the vertical distance from the base line and may be approximated by directly reading the chart.

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